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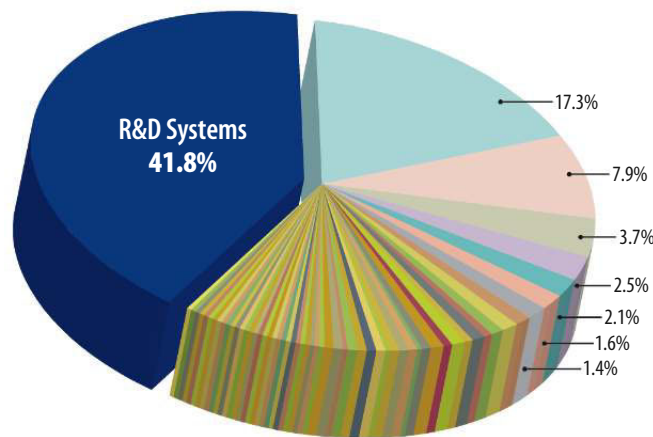
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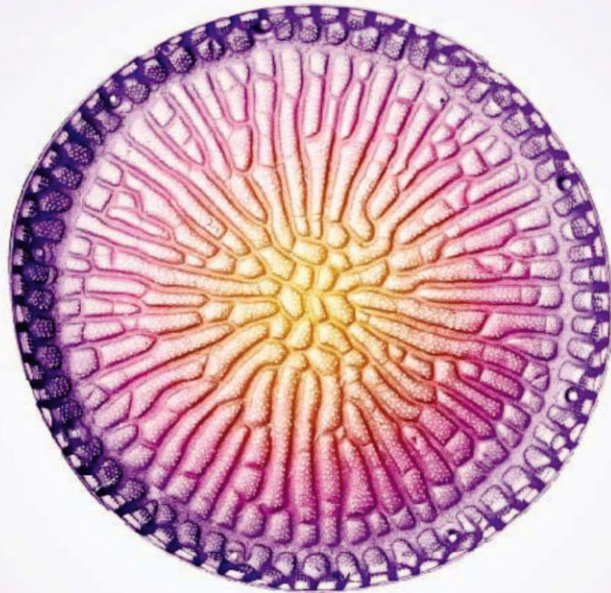
COVER

False-colored scanning electron micrograph of the bacterial pathogen *Streptococcus pneumoniae* (typical length, ~1 micrometer), a major cause of pneumonia and meningitis. On page 430, Croucher *et al.* describe the use of whole-genome sequencing to track the global spread of a rapidly recombining *S. pneumoniae* lineage as it adapts to antibiotic use and vaccine introduction.

Image: Dennis Kunkel Microscopy, Inc./Visuals Unlimited, Inc.

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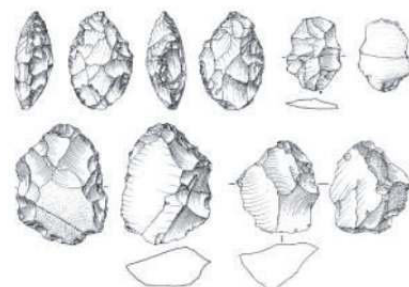
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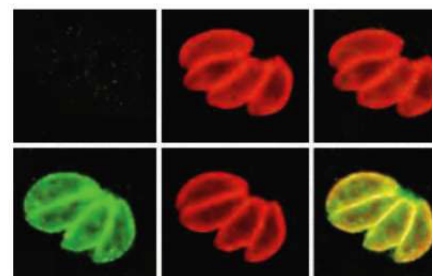
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A woman with dark hair, wearing a white lab coat over a red top and black leggings, is running towards the viewer with a joyful expression. She is holding two small, rectangular microarray chips, one in each hand. The background is a bright, clean white, featuring a large, stylized DNA double helix structure composed of golden-yellow lines and text representing genetic code. The overall image conveys a sense of dynamic progress and scientific achievement in genomics.

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Microtubule Stabilization Reduces Scarring and Causes Axon Regeneration After Spinal Cord Injury

F. Hellal et al.

Taxol stimulates the capacity of axons to grow after spinal cord injury.

10.1126/science.1201148

Structures of SAS-6 Suggest Its Organization in Centrioles

M. van Breugel et al.

Self-assembly of a centriolar protein may contribute to organizing the cartwheel-like hub and establishing centriole symmetry.

10.1126/science.1199325

The Magnitude and Duration of Late Ordovician–Early Silurian Glaciation

S. Finnegan et al.

Carbonate isotopes reveal a link between past ocean temperatures and mass extinction.

10.1126/science.1200803

Linking Policy on Climate and Food

H. C. J. Godfray et al.

10.1126/science.1202899

SCIENCE NOW

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Highlights From Our Daily News Coverage

Frédéric Chopin's 'Madness' Diagnosed

New study argues that composer suffered from a type of epilepsy.

<http://scim.ag/mad-chopin>

The World's Smallest Farmers

Single-celled amoebas plant bacteria for food.

<http://scim.ag/tiny-farmers>

When Trees Attack, Fungus Can Parry

Genetic analysis reveals why the pine beetle has ravaged so many trees.

<http://scim.ag/Grosmannia>

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25 January issue: <http://scim.ag/ss012511>

RESEARCH ARTICLE: Genome-Wide RNAi Screen Reveals Disease-Associated Genes That Are Common to Hedgehog and Wnt Signaling

L. S. Jacob et al.

Loss of the kinase and tumor suppressor Stk11 (Lkb1) has opposite effects on Hh and Wnt signaling.

RESEARCH ARTICLE: V₂ Receptor–Mediated Autocrine Role of Somatodendritic Release of AVP in Rat Vasopressin Neurons Under Hypo-Osmotic Conditions

K. Sato et al.

The antidiuretic hormone arginine vasopressin (AVP) is also an autocrine signal that functions in cell volume regulation.

RESEARCH ARTICLE: Rho and Rho-Kinase Activity in Adipocytes Contributes to a Vicious Cycle in Obesity That May Involve Mechanical Stretch

Y. Hara et al.

Mechanical stretch activates Rho-kinase in adipocytes, promoting obesity and obesity-related complications.

PODCAST

Researchers discuss how mechanical stretch regulates signaling in adipocytes and in hypothalamic neurons.

MEETING REPORT: Dependence Receptors—From Basic Research to Drug Development

P. Mehlen and D. E. Bredesen

PERSPECTIVE: Control of DNMT1 Abundance in Epigenetic Inheritance by Acetylation, Ubiquitylation, and the Histone Code

C. Bronner

JOURNAL CLUB: The Role of L(u)ck in T Cell Triggering

R. Berry

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Science Blogging and Tenure

V. Raper

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<http://scim.ag/dFDgml>

A Loyal Fan of Women's Health Research

K. Hede

Physician-scientist Rebecca Jackson's enthusiasm for research is matched only by her passion for Ohio State football.

<http://scim.ag/hPH8Nj>

Experimental Error: Lies, Damned Lies, and Seminars

A. Ruben

For all the naive and gullible graduate students out there, here is a handy guide to what those speakers are really saying.

<http://scim.ag/ezFYgK>

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Integrating Medicine and Science

26 January issue: <http://scim.ag/stm012611>

COMMENTARY: Between Confidentiality and Scientific Exchange—The Place of Publication in Drug Discovery and Pharmaceutical Research

M. Clozel

A combined preclinical and clinical publication might be the ideal approach in translational medicine.

RESEARCH ARTICLE: Uroporphyrinogen Decarboxylase Is a Radiosensitizing Target for Head and Neck Cancer

E. Ito et al.

Uroporphyrinogen decarboxylase inhibition sensitizes head and neck cancer to both radiotherapy and chemotherapy.

RESEARCH ARTICLE: Manipulating the Bioenergetics of Alloreactive T Cells Causes Their Selective Apoptosis and Arrests Graft-Versus-Host Disease

E. Gatz et al.

Bioenergetic properties differentiate alloreactive T cells from other proliferating cells and can be exploited to arrest GVHD in mice.

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Science Policy News and Analysis

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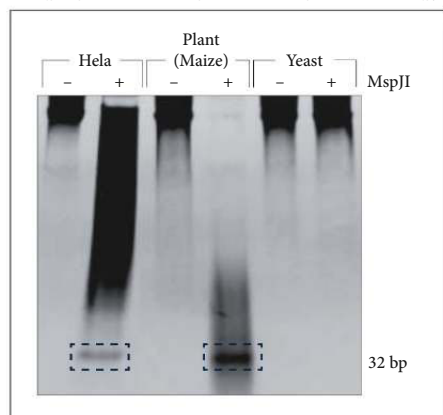
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Digesting Grass

Identification of additional enzymes that can degrade cellulose efficiently should help in the development of biofuels on an industrial scale. Uncultured microorganisms living in cow rumen are highly effective at degrading plant cell walls. **Hess *et al.*** (p. 463) used metagenomics and single-genome sequencing to assemble draft genomes from microbes adhering to rumen-incubated switchgrass to identify nearly 28,000 genes related to known biomass-degrading families. Ninety candidate carbohydrate-degrading enzymes were synthesized and their activity analyzed against 10 different substrates, including the biofuel crops miscanthus and switchgrass. The data set greatly expands the repertoire of full-length genes available for use in industrial biotechnology.

Nickels High and Low

Certain transition metal complexes undergo a phenomenon termed spin crossover, in which a rise in temperature shifts the proportion of valence electrons that share the same spin orientation, and thus drive magnetic response. However, the collective shift across an ensemble of molecules in solution tends to be gradual and has been difficult to harness in switching applications such as contrast variation in magnetic resonance imaging. **Venkataramani *et al.*** (p. 445) present an alternative magnetic switching scheme, in which a photoresponsive ligand is used to modulate the electronic structure of a nickel complex. Isomerizations driven by different wavelengths of light selectively push the ligand onto or off the nickel ion, thereby inducing a high or low spin state. Up to 75%

of the ensemble could thus be switched rapidly between magnetically active and passive states at room temperature.

Ecology ↔ Evolution

Ecology and evolution are fundamentally connected—ecological processes drive patterns of natural selection and thus shape evolutionary trajectories. The possibility that evolutionary change could also influence ecological processes is much less clear; it has often been assumed that evolutionary change takes too long to impact ecology. **Schoener** (p. 426) reviews how evolutionary change can in fact be extremely rapid, occurring over ecological time scales. Rapid evolutionary changes can thus give feedback on ecology and impact ecological processes such as population growth, community structure, and productivity.

The Pneumococcus Game

Pneumonia and meningitis caused by *Streptococcus pneumoniae* have accounted for millions of deaths through the ages. The Spanish strain is a globally occurring, multiply antibiotic-resistant lineage that has been exceptionally troublesome. Isolates of this lineage have been collected since 1984 from diverse geographic origins. **Croucher *et al.*** (p. 430, see the cover; see the Perspective by **Enright and Spratt**) have now used high-throughput sequencing technology to dissect serotype 23F's detailed evolution. The bacterium appears to respond to public health measures with extraordinary genetic agility, using a variety of recombination and base substitutions that can turn over three-quarters of the genome, enabling the pathogen to evade the effects of vaccines and antibiotics.

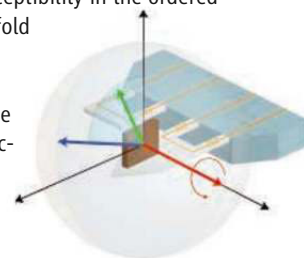
Decoding a Childhood Cancer

The identification of recurrent genetic changes in human tumors can provide important mechanistic insights into how tumors arise and, ideally, prompt new ideas for effective therapies. To date, this “cancer genomics” strategy has been applied only to adult cancers. **Parsons *et al.*** (p. 435, published online 16 December) now catalog the genetic alterations present in medulloblastoma, a brain tumor that mainly affects children. Interestingly, there were 5 to 10 times fewer genetic alterations in these tumors compared with solid tumors that typically affect adults. Among the most frequently

mutated genes were two coding for enzymes that methylate histones, as well as genes affecting signaling pathways critical for normal brain development.

Understanding Mysterious Order

The heavy fermion compound URu_2Si_2 enters an ordered phase below 17.5 kelvin but, despite 25 years of extensive study, the exact nature of this phase remains a mystery. Using very pure, small specimens, **Okazaki *et al.*** (p. 439) found that the magnetic susceptibility in the ordered phase breaks the fourfold rotational symmetry of the URu_2Si_2 crystal, which suggests that the hidden order is an electronic nematic state.



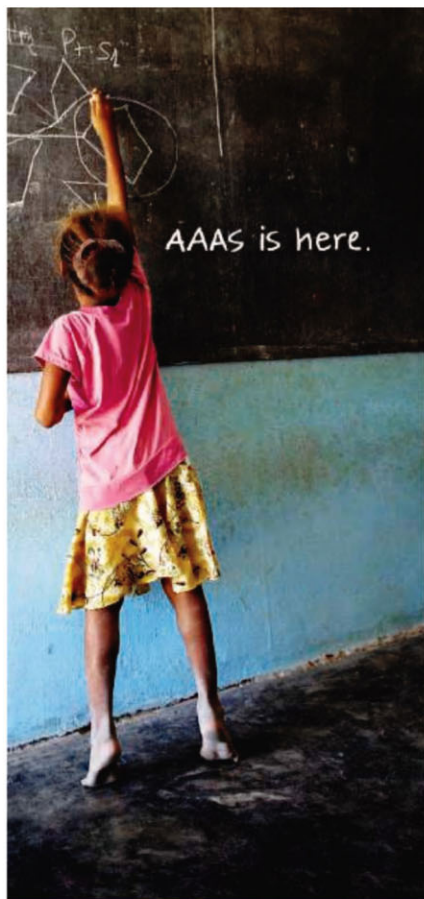
Laser Backfire

Standoff detection is an important method to monitor or probe regions that are otherwise inaccessible to direct sampling techniques. **Dogariu *et al.*** (p. 442) report the observation of high-gain infrared lasing in air from the focal region of an ultraviolet laser. The backward-directed infrared laser light could then be used as a remote spectroscopic tool that samples the air on its return path back to the sender position. The technique should find a range of applications—from the detection of greenhouse gas emissions and pollution to the detection of potentially hazardous and explosive materials.

Reaction of Hydrogen Isotopes, Great and Small

Kinetic isotope effects reveal mechanistic insight into chemical reactions. Larger isotope ratios often lead to larger changes in rate, and, commonly, the largest ratio is 2—for the substitution of deuterium for hydrogen. **Fleming *et al.*** (p. 448; see the Perspective by **Alexander**) compared results for the simplest chemical reaction, of an H atom with H_2 , for two hydrogen isotopes created with positive and negative muons, which provide an unprecedentedly large mass ratio of 36. At 500 kelvin, the relative reaction rates measured agreed with those calculated using variational transition state theory.

Continued on page 376



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This Week in *Science*

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Warmest in 2000 Years

Although the climate warming of the past century has occurred nearly everywhere, the Arctic shows the greatest temperature increase. Most of the heat transported to the Arctic comes from the Atlantic Ocean, which has warmed greatly over the past 150 years. How did the temperature of Arctic inflow from the Atlantic vary before anthropogenic climate warming began? **Spielhagen *et al.*** (p. 450) present a record of oceanic temperature variations for the last 2000 years, which shows that Atlantic water entering the Arctic through the Fram Strait is warmer than it has been in two millennia. This unprecedented warming is likely to represent a key factor in the apparent transition toward an ice-free Arctic Ocean.

Before the Mediterranean Exodus

Modern humans originated in Africa and then spread to Eurasia and beyond. The timing and locations of their emigration have been uncertain; genetic and archaeological data support an exodus along the Mediterranean by 60,000 years ago, but earlier attempts may have occurred, for example, in response to the massive Toba volcanic eruption about 75,000 years ago. **Armitage *et al.*** (p. 453; see the news story by **Lawler**) now describe artifacts from about 100,000 years ago found in eastern Arabia, indicating that modern humans were already there by then. This location would have allowed access to the Fertile Crescent and India as sea level dropped. The findings suggest that there may indeed have been an early exodus of modern humans from Africa.

Predatory Synergism

Synergies between species, in which the combined effect of multiple species is greater than the sum of their individual effects, link biodiversity to ecosystem function, but these synergies may be sensitive to external influences. **Piovia-Scott *et al.*** (p. 461) investigated how a synergy between two very different predator species in the Bahamas was affected by the influx of resources from the adjacent ocean. Adding seaweed to entire small islands (simulating the natural marine-to-terrestrial energy transfer that occurs during storms) eliminated the synergistic effect of two predators—lizards and ants—on arthropod herbivores and their food plants, increasing the overall rate of herbivory.



Parasite Replication Trigger

Apicomplexan parasites, including *Toxoplasma gondii* and the malaria parasite *Plasmodium falciparum*, actively invade host cells. Little is known about the signals that govern initiation of replication once the parasite is intracellular. Apical membrane antigen 1 (AMA1) is released onto the parasite surface during invasion and cleaved by intramembrane proteolysis, mediated by a rhomboid-like serine protease, ROM4. **Santos *et al.*** (p. 473, published online 23 December; see the Perspective by **Cowman and Tonkin**) used conditional expression of *Toxoplasma* ROM4 to show that ROM4 activity is not essential for invasion, but instead is required for subsequent replication of the intracellular parasite. Furthermore, transgenic expression of the cleaved cytoplasmic tail of AMA1 alone—either from the *Toxoplasma* AMA1 or from its *P. falciparum* ortholog—completely restored the replicative capacity of the intracellular parasites. Thus, intramembrane cleavage of AMA1 is required to trigger parasite replication within the host cell.

Bigger Beats Smaller

Social cooperation has been the subject of intense experimental investigation, both in humans and in other animals, and recent studies have detailed the developmental origins of these representations in human children and infants. **Thomsen *et al.*** (p. 477) now document the appearance of social dominance awareness in infants before the end of the first year. Infants were found to be capable of representing the conflicting goals of two cartoon characters, appearing to use size as a cue to predict which character will win.

CREDIT: PIOVIA-SCOTT ET AL.



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New Views on News and Research

THIS WEEK, *SCIENCE* UNVEILS TWO PROJECTS AIMED AT MAKING OUR CONTENT MORE USEFUL and accessible amid today's information glut: a revamp of the News section to provide a faster launch into the week's top stories, and a prototype of some new ideas for our online articles, on which we seek your feedback.

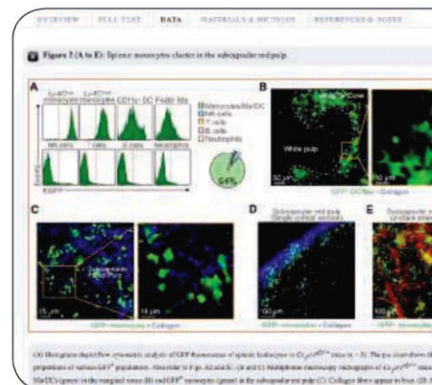
For 20 years, *Science*'s News section has included a page called Random Samples, an eclectic mix of brief items about science and the scientific community. Reader surveys have consistently shown that it is one of the most-read features in the magazine. Starting with this issue (see p. 382), we are expanding and refocusing the type of coverage that has made Random Samples so popular. Each week, the News section will open with three or four pages of brief items under the heading News of the Week. The aim is to give busy readers a quick take on the important events in the world of science. It will include a roundup of the top stories in science policy, written from our far-flung bureaus: Washington, DC; Cambridge, UK; Paris; Berlin; Tokyo; Beijing; New Delhi; and São Paulo. We will bring you news of scientists making headlines and present the bottom line on newly published research papers. In the tradition of Random Samples, we will also include items that are offbeat, whimsical, or just plain interesting—quotes, factoids, striking images. News of the Week will be edited by Lauren Schenkman, who has been editing Random Samples since the tragic death last year of the page's long-time editor Constance (Tancy) Holden. The items will be drawn in part from *Science*'s daily online coverage of research findings, *ScienceNOW*, and our science policy blog *ScienceInsider* (<http://news.sciencemag.org>).

News of the Week is designed to provide a quick overview of the week's top events; the rest of the News section focuses beyond the headlines. In a section renamed News & Analysis, our award-winning news team will draw on expert opinion to put the news in context. And the News Focus section will continue to take a broad look at the trends, personalities, and events shaping the world of science. At a time when traditional journalism is under severe pressure, and science reporting is declining in many major media outlets, our goal—and that of our publisher, the American Association for the Advancement of Science—is to provide the best coverage across the sciences and throughout the interface of science with society.

On a different note, we're also reaching out to our Web readers this week for their fresh, direct input to help us rethink the format and functionality of our online articles. Recently, a staff team from across *Science* has been exploring a variety of new ideas for Web article content, including a tabbed interface, summary material to help put the article in context, more visible ties to related content, a different treatment for figures and supporting online data, and a variety of other possibilities that we hope will save users time, make online articles more readable and functional, and open up new possibilities for further exploration.

We've rolled these ideas into an initial prototype that is posted in a beta version at <http://labs.sciencemag.org/>. We encourage readers to try out the prototype and leave us their thoughts. What do you like and dislike about this first model? What's missing that would help you work more efficiently with the content; and what's distracting? What's at the top of your own "wish list" for more effectively interacting with scientific content? The prototype is very much "release 0.1"; we plan to incorporate new ideas and adjustments based on your feedback. This process will help us to zero in on a new article interface that's better tuned to user needs in the crowded and rapidly changing environment for communicating scientific information. Stop by for a look, give us your thoughts and ideas, and become a part of this new undertaking.

— Colin Norman and Stewart Wills



SOCIOLOGY

Complex Contributions

It is well established that academic achievement is related to socioeconomic status (SES), but it is not yet clear at what age this effect emerges. Tucker-Drob *et al.* examined whether the effects of SES on mental ability could be observed in infancy. They analyzed 750 pairs of twins from the Early Childhood Longitudinal Study, Birth Cohort data file. At 10 months and 2 years of age, the children's mental ability was assessed using tests that included pulling a string to ring a bell, putting cubes in a cup and matching pictures, among other tasks. SES was determined from the education and occupations of the parents and family income.

At 10 months of age, SES was not related to mental ability. Between 10 months and 2 years of age, however, the authors observed that increased SES was associated with larger gains in mental ability. At 2 years of age, the influence of genetics on cognitive development was higher in children of higher SES, whereas genetics had very little effect on the mental ability of children raised in low-SES homes. These findings do not indicate any differences in intrinsic intelligence, but may provide support for efforts to provide enrichment for young children from disadvantaged backgrounds. — BJ

Psychol. Sci. **22**, 125 (2011).

SIGNALING

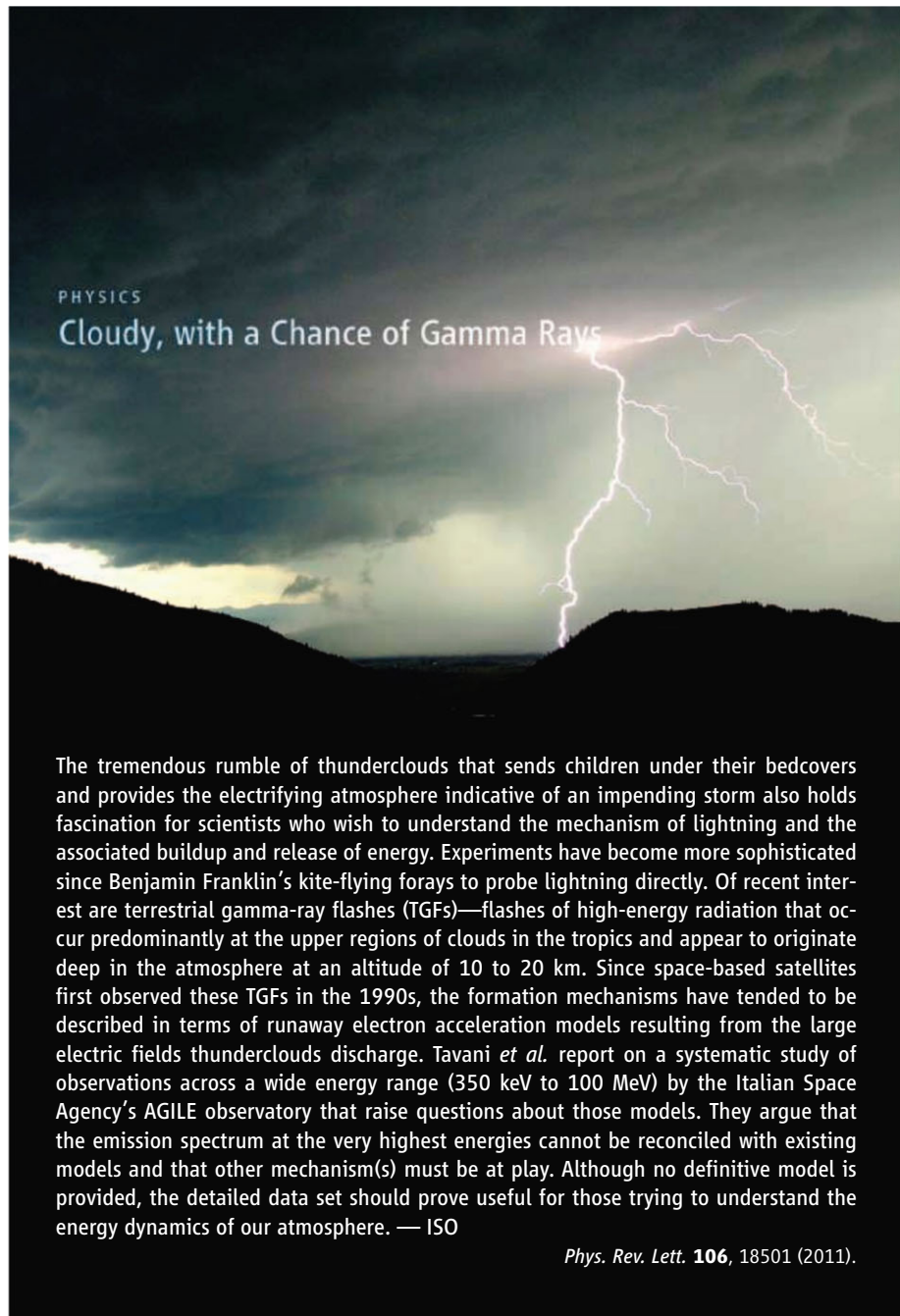
Signaling Across Pathways

The signaling pathways that regulate cell function are obvious targets for therapeutic intervention, but such strategies are complicated by our incomplete understanding of these pathways and also the links between them. Jacob *et al.* used an RNA interference screen in mouse cells to find new components of the Wnt and Hedgehog signaling pathways. Both are key regulatory pathways in development that are also linked to various diseases, including cancer. Loss of the protein kinase Lkb1 inhibited Hedgehog pathway-dependent gene expression through effects on the primary cilium, where Hedgehog signaling is organized. Loss of Lkb1 also disrupted Wnt signaling, but through a

distinct mechanism. Similar results were observed when expression of Lkb1 was inhibited in zebrafish embryos. Similar expanded approaches thus

appear necessary to understand the functions of key signaling components and how they might be managed to affect complex diseases. — LBR

Sci. Signal. **4**, ra4 (2011).



PHYSICS

Cloudy, with a Chance of Gamma Rays

The tremendous rumble of thunderclouds that sends children under their bedcovers and provides the electrifying atmosphere indicative of an impending storm also holds fascination for scientists who wish to understand the mechanism of lightning and the associated buildup and release of energy. Experiments have become more sophisticated since Benjamin Franklin's kite-flying forays to probe lightning directly. Of recent interest are terrestrial gamma-ray flashes (TGFs)—flashes of high-energy radiation that occur predominantly at the upper regions of clouds in the tropics and appear to originate deep in the atmosphere at an altitude of 10 to 20 km. Since space-based satellites first observed these TGFs in the 1990s, the formation mechanisms have tended to be described in terms of runaway electron acceleration models resulting from the large electric fields thunderclouds discharge. Tavani *et al.* report on a systematic study of observations across a wide energy range (350 keV to 100 MeV) by the Italian Space Agency's AGILE observatory that raise questions about those models. They argue that the emission spectrum at the very highest energies cannot be reconciled with existing models and that other mechanism(s) must be at play. Although no definitive model is provided, the detailed data set should prove useful for those trying to understand the energy dynamics of our atmosphere. — ISO

Phys. Rev. Lett. **106**, 18501 (2011).

ASTROPHYSICS

Tuning in to Bright Sources

The Fermi space telescope has revealed thousands of new sources of gamma-ray emission; however, the nature of hundreds of them remains a mystery. To check whether some of the unidentified sources could be pulsars, Ransom *et al.* searched for radio pulsations in 25 sources in the Fermi Large Area Telescope Bright Source List—a catalog of the brightest sources detected in the early months of the Fermi mission. These 25 sources

CREDITS (TOP TO BOTTOM): ISTOCKPHOTO.COM; JACOB ET AL., *SCI. SIGNAL.* **4**, RA4 (2011)

were selected to be nonvariable and not associated with known pulsars, black holes at the centers of galaxies, or x-ray sources that had previously been searched for radio pulsations. Radio observations with the Green Bank Telescope revealed three pulsars with millisecond periods, all of which are in binary systems where a neutron star and a companion star orbit one another about their common center of mass. The new pulsars have properties consistent with those of typical radio millisecond pulsars. Their detection suggests that most, if not all, radio millisecond pulsars produce gamma rays, and that their radio and gamma-ray beams are comparable in size. It is thus possible that millisecond pulsars contribute to the diffuse isotropic gamma-ray background. — MJC

Astrophys. J. **727**, L16 (2011).

CHEMISTRY

Carbonic Acid Aloft

The chemistry of carbon dioxide in water plays a remarkably diverse series of roles in our daily lives—modulating acidity in blood and ocean water, lending soda its sparkle and bread its fluff. It's all the more remarkable, then, that the adduct of the two molecules, carbonic acid, spent centuries eluding characterization. Its deprotonated conjugate bicarbonate (HCO_3^-) is reactive but easily isolable; in contrast, it was only recently established that the HOC(O)OH molecule persists for any length of time in solution before breaking apart. Bernard *et al.* have now shown that carbonic acid is also at least kinetically stable in the gas phase as well. By subliming the solid acid and then capturing it in a frozen argon matrix, the authors were able to detect the vibrational signatures of two conformational isomers (with a W-shaped one predominating) as well as a hydrogen-bonded dimer. The findings raise the prospect of finding the molecule in comet tails or other planetary atmospheres. — JSY

Angew. Chem. Int. Ed. **10.1002/anie.201004729** (2010).

SIGNALING

Working Against the Clock

Our circadian rhythms keep us in tune with the day. Some of the molecular signals that implement and regulate the circadian rhythm have been identified, but the complexity of the various systems affected by circadian rhythms is not well understood. Many people work against their circadian clock, whether it be a scientist who hops seven time zones eastward and still hopes to be awake at a conference session with the lights dimmed, or someone who works the night shift and needs cognitive acuity and physical dexterity during the hours when most of us are asleep. To

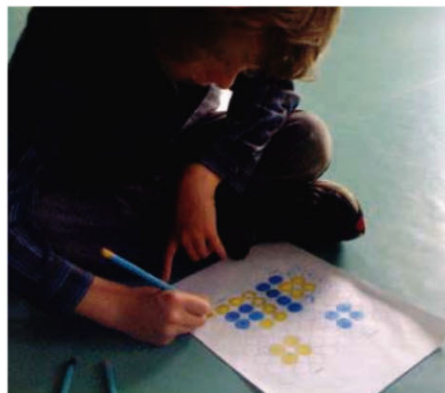
further understand how disruption of the circadian rhythm affects us, Karatsoreos *et al.* studied mice kept in an unnaturally short day/night cycle. With a cycle of 20 rather than 24 hours, the mice showed a variety of disrupted responses. They gained weight, had elevated insulin levels, and demonstrated reduced cognitive flexibility. Neurons in the brain's cortex showed reduced complexity. Study of these mice may help us understand, for example, the unexpected incidence of obesity among shift workers. — PJH

Proc. Natl. Acad. Sci. U.S.A. **108**, 10.1073/pnas.1018375108 (2011).

EDUCATION

Child Scientists

What would happen if, instead of consulting previous literature, scientists asked children for advice on designing experiments? In the case of the Blackawton Bees, 8- to 10-year-old children capitalized on their own curiosity and observations to devise questions, propose a hypothesis, design experiments, and perform data analysis in an original study examining how bees perceive and remember their surroundings. Besides



discovering that bees use both color and spatial analysis in deciding which color of flower to forage from, it served as an example of real science and engaged the students. This is evident in the published paper, written by the students, that contains statements such as "Before doing these experiments we did not really think about bees," and "This experiment is important, because no one in history, including adults, has done this experiment before." In this way, science education became more of a process of contributing to asking questions and devising strategies to answer those questions instead of a passive classroom lesson. Afterward, the students came to the same conclusion that every scientist has come to at one point in their career: "Science is cool and fun because you get to do stuff that no one has ever done before." — MM

Biol. Lett. **10.1098/rsbl.2010.1056** (2010).

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Science Careers

From the journal *Science*



National Academy of Sciences Congratulates the **RECIPIENTS OF ITS 2011 AWARDS**

Since 1886 the National Academy of Sciences has presented awards to honor outstanding contributions to science and to encourage new and innovative research.

NAS Public Welfare Medal

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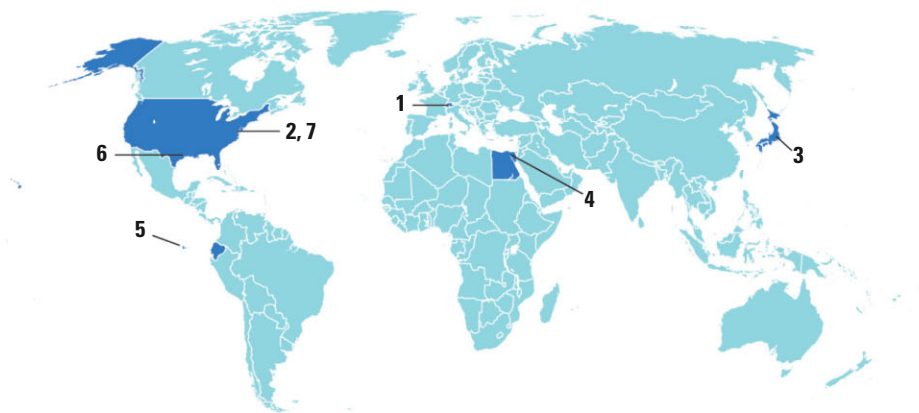
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University of California, San Francisco

Nominations for the 2012 awards will be accepted through September 15, 2011. For details visit:

www.nasonline.org/awards



NATIONAL ACADEMY OF SCIENCES



AROUND THE WORLD

Basel, Switzerland **1**

Melanoma Drug Extends Life

The drug company Roche announced that an experimental drug increased the survival of patients with advanced skin cancer whose tumors had a specific mutation in a gene called *B-RAF*. RG7204, developed by the biotech company Plexxikon, targets a protein encoded by the mutated gene that drives cancer growth in about half of all patients with malignant melanoma. About 40,000 people worldwide die annually from this aggressive cancer, most less than a year after being diagnosed.

In the closely watched phase III trial, patients with the *B-RAF* mutation who received the drug lived significantly longer and their tumors grew more slowly compared with patients who received a standard chemotherapy drug, Roche officials announced in a press release. But the company did not disclose how much longer patients lived on average. Full results will be reported later this year at a meeting. Patients receiving the standard drug will now be offered the option of switching to RG7204.

Washington, D.C. **2**

JPL Scientists Subject To Background Checks

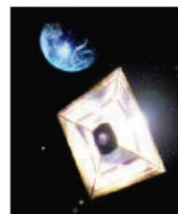
The U.S. Supreme Court has upheld the use of background checks by the government on scientists and other workers at NASA's Jet Propulsion Laboratory (JPL). The decision caps a legal battle begun in 2007, when 28 scientists and engineers at JPL, which is owned by NASA but operated by the California Institute of Technology in Pasadena, filed suit against new screening procedures

announced by NASA. They argued that questionnaires asking about drug use, counseling, and "trustworthiness" were too intrusive and harmed the open environment at JPL. As contract employees, they said, they should be subject to less scrutiny than government employees who work with classified material. An appeals court agreed, but the Supreme Court unanimously disagreed.

NASA will now have to decide whether to reinstate the policy, which has been on hold since 2007. <http://scim.ag/NASA-checks>

Tokyo, Japan **3**

Solar Sail Mission Sails On



The IKAROS solar sail mission was formally extended, having passed all performance tests planned for its original 6-month life. Launched by the Japan Aerospace Exploration Agency (JAXA) last year on 21 May (*Science*, 7 May 2010, p. 677), IKAROS, with its 20-meter diagonal, 0.0075-millimeter-thick polyimide sail, became the first craft propelled through space by the pressure of photons in sunlight. "We achieved complete success," says mission manager Osamu Mori of JAXA's Institute of Space and Astronautical Science in Sagami-hara, near Tokyo. JAXA will fund the mission for another year so scientists can try to pull off some advanced navigational tricks, such as varying the sail's angle to the sun. Such experiments will help in planning more ambitious solar sail missions, says Mori. Separately, NASA announced last week that its solar sail mission, NanoSail-D, which was thought to be malfunctioning, successfully deployed its sail and is working as planned.

Cairo, Egypt **4**

Dead Queen Still Kicking Up Dust

Egypt's Supreme Council of Antiquities demanded the return of a famous bust of Queen Nefertiti that has been in Berlin's Neues Museum since its discovery in 1912 by German archaeologists. Egypt has been trying to get her back since the 1920s—Adolf Hitler refused to send her back in the 1930s—but now council chief Zahi Hawass has made the request in writing to the Prussian Cultural Heritage Foundation, which oversees the museum. Egypt maintains that the discoverer misled Egyptian authorities after its discovery, but the initial German reaction was dismissive.



Galápagos Islands, Ecuador **5**

Fight Rages On Against Invasive Rats

Conservationists have stepped up their war against alien rats in the Galápagos. Officials with Ecuador's Galápagos National Park announced that with conservationists from various nonprofit organizations, they had begun carpet-bombing the archipelago's smaller islands with rat poison systematically released from a helicopter. Rats first arrived as stowaways in Western sailing ships and are a problem because they eat native tortoise and bird eggs. Although conservationists have been killing rats for years using bait and traps, the new strategy aims for "100% eradication" from nine islands and islets, including Jervis and Beagle islands, the officials announced in a statement. To protect a native bird that might

NOTED

>The **Kafkaesque bureaucracy of the Framework Programme**, the European Union's multibillion-euro research funding system, **is getting simpler**. A specific E.U.-approved accounting system will no longer be required, small business partners can now be paid a flat rate, and multiple sets of rules and procedures will be eliminated. <http://scim.ag/no-kafka>

otherwise eat the poisoned rats, conservationists captured 20 Galápagos hawks and plan to keep them in captivity for 2 months. They said that “mitigation steps will be taken” to protect the sole endemic rodent, a mouse found on Santiago Island.

Houston, Texas 6

Cancer Center Receives Big Thank-You Gift

When MD Anderson Cancer Center in Houston, Texas, admitted some cancer patients from the United Arab Emirates (UAE) for treatment, officials there had no idea it would lead to the largest gift in the hospital's history. Now, the Khalifa bin Zayed Al Nahyan Charity Foundation, formed by (and named after) the UAE's president, has announced it is giving MD Anderson \$150 million. The money will be used for a new building and work in pancreatic cancer and personalized medicine.

Some \$25 million will go to study biomarkers, genetic patterns in tumors that could guide treatment for individual patients. “There’s been a lot of stops and starts” in the biomarkers field, with many not panning out, admits Raymond DuBois, the cancer center’s provost and executive vice president. “We’re at a critical stage now,” and this infusion of money, he says, will surely help.

Washington, D.C. 7

A Call for Stem Cell Researchers to Share

Researchers working with stem cells should follow the example of their colleagues in genetic sequencing and clinical research, setting up global networks for sharing data, materials, and intellectual property, according to a report released by an international consortium on stem cells and ethics. The Hinxton Group recommended setting up a publicly available global stem cell registry that would include a cell line’s characteristics and information on how it was derived. Stem cell banks and cell repositories should be expanded and should coordinate their work, the report says, and funding agencies and journals should make data and material sharing mandatory. A database of stem cell-related patents is also urgently needed, the group says, to help scientists deal with the thorny thicket of intellectual property that has grown along with the hot field.

<http://scim.ag/hinxton-group>

NEWSMAKERS

Japan Prizes

TOKYO—A breakthrough drug and a computer operating system shared the laurels of one of science’s top honors, the Japan Prize.



Clockwise from top left: Kishimoto, Hirano, Ritchie, and Thompson.

The Bioscience and Medical Science prize goes to Tadamitsu Kishimoto and Toshio Hirano, both of Osaka University, for identifying the protein interleukin 6, elucidating its immune functions, and determining its role in rheumatoid arthritis. The pair

ultimately helped develop a drug that treats the debilitating disorder by blocking the protein’s activity.

Kenneth Thompson and Dennis Ritchie take home the Information and Communications prize for developing the UNIX computer operating system in the 1960s and ’70s while at Bell Laboratories in Murray Hill, New Jersey. UNIX set new standards for simplicity and ease of adapting software to different computing platforms. The source code was also distributed to users so that they could contribute improvements, marking the beginning of the open systems concept. Thompson is now at Google Inc.; Ritchie is retired.

The winners in each category will share \$600,000 and receive commemorative medals at an April ceremony here.

<http://scim.ag/japan-prize>

THEY SAID IT

This week, our Facebook fans chimed in on a story of a dino with just one finger (see p. 384). Some of our favorite comments:

“Thanks for flipping us the prehistoric bird!”

“It’s ok as long as the dino doesn’t have to do math homework.”

“And that was how the binary system was created, right?”

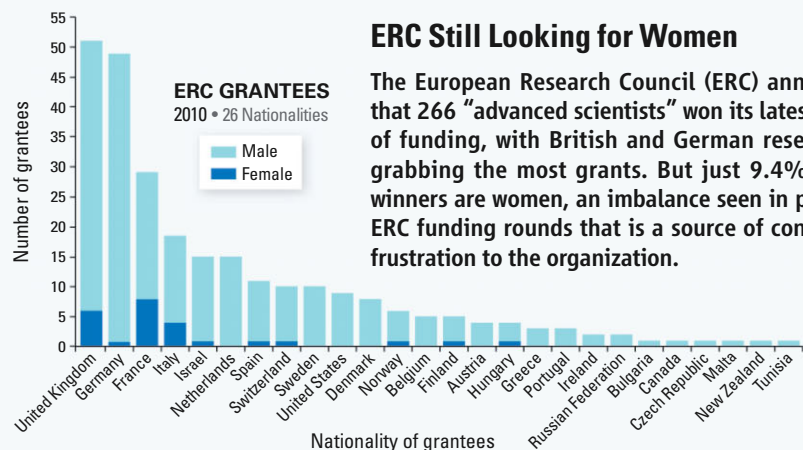
<http://www.facebook.com/ScienceNOW>



Finn Bags Swedish Prize

This year’s Crafoord Prize has gone to Finnish ecologist Ilkka Hanski of the University of Helsinki for his contributions to understanding the impact of habitat fragmentation on species’ survival. Hanski has spent much of his 30-year career assessing the risk of local extinctions in environments subject to growing human influence. The prize, awarded annually by the Royal

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ERC Still Looking for Women

The European Research Council (ERC) announced that 266 “advanced scientists” won its latest round of funding, with British and German researchers grabbing the most grants. But just 9.4% of the winners are women, an imbalance seen in previous ERC funding rounds that is a source of continuing frustration to the organization.

>>NEWSMAKERS

Swedish Academy of Sciences to researchers in fields not covered by the Nobels, recognizes Hanski for developing models to help conservationists manage natural environments.

Hanski says he has not yet decided what he will do with the 4 million kronor (approximately \$600,000) that come with the prize, but he suspects that he will buy a plot of forest in Finland to save it from future development. <http://scim.ag/crafoord>

Canadian-Born Geologist Tapped for Key E.U. Research Post

Experimental volcanologist Donald Bruce Dingwell was born and raised in Canada, but he may soon be playing a big role in European research funding. *Science* has learned that he has been chosen as the next secretary general of the European Research Council (ERC), the E.U.'s funding agency for individual basic researchers. But negotiations about his contract are still ongoing, cautions ERC President Helga Nowotny.

Dingwell is currently director of the Department of Earth and Environmental Sciences at Ludwig Maximilians University Munich in Germany. As secretary general, he would be a liaison to ERC's executive agency, a managing body ultimately controlled by the European Commission, on behalf of ERC's scientific council, which sets strategy. ERC previously decided to scrap the position of secretary general but reversed that decision pending an overhaul of its organizational structure. <http://scim.ag/dingwell>



FINDINGS

Star Light, Star Bright

This infrared image from NASA's Spitzer Space Telescope shows what may be the most luminous group of superstars in the entire galaxy. Such groups—named OB associations for the O and B spectral types of their hot, blue suns—sculpt vast regions of space through their radiation and supernova explosions. The newly discovered Dragonfish association in the Southern Cross is 32,000 light-years distant and harbors about 400 hot, blue, luminous stars. Mubdi Rahman of the University of Toronto in Canada and colleagues will report in an upcoming issue of *The Astrophysical Journal Letters*. The stars' extreme ultraviolet radiation strips electrons from protons, thereby ionizing interstellar hydrogen gas and setting it aglow.

Let's Stay Together

Parting is such sweet sorrow that Sumatran and Bornean orangutans may have separated into distinct species more than half a million years later than previously assumed.

Researchers have completed a draft sequence of the orangutan genome derived

from 11 individuals on the islands of Sumatra and Borneo, the only places where our endangered, orange-haired relatives live in the wild. A comparison of the two species' DNA suggests they separated just 400,000 years ago, revising previous estimates of at least 1 million years ago. (They were physically separated at least 21,000 years ago, when land bridges between the two islands disappeared.) "Most previous studies used small sets of markers and a limited amount of DNA sequence," says Devin Locke, a structural geneticist at Washington University School of Medicine in St. Louis, Missouri, and the lead author of the study, which appears online this week in *Nature*.

Orangutans lead a more sedentary lifestyle than other great apes, and their DNA suggests they may evolve more slowly, too. Key drivers of evolution are stretches of DNA called retrotransposons that jump around the genome, creating new genes or altering regulation of existing ones. The new data reveal that retrotransposons known as Alu elements have moved around the orangutan genome much less than they have in the human and chimpanzee genomes (the only other two great apes to have been sequenced). <http://scim.ag/orang-genes>



Single-Digit Dino

Meat-eating dinosaurs were very good at finding food, thus their evolutionary success over some 165 million years. But during their time on Earth, they kept losing something that might seem important: their fingers. The earliest carnivorous dinosaurs had five fingers, although only four were actually functional. Many later meat eaters had three, and evolution left the mighty *Tyrannosaurus rex* with only two. Now researchers have unearthed the first known dinosaur with only one finger. The new single-digit species, named *Linhenykus monodactylus*, was found in a roughly 80-million-year-old rock formation in Inner Mongolia, lead author Xing Xu of the Chinese Academy of Sciences Institute of Vertebrate Paleontology and Paleoanthropology in Beijing and colleagues report online in the *Proceedings of the National Academy of Sciences*.

Linhenykus, which was probably about a meter tall, belongs to a family of dinosaurs called alvarezsauroids. The team suggests that the single claw-like digit was an adaptation for digging, perhaps for insects such as termites.

BY THE NUMBERS

40% The percentage of U.S. high school seniors who scored below the basic level of achievement in science on the 2009 National Assessment of Educational Progress. Only 1% of 12th-graders scored at the advanced level on the test, a quadrennial measure of reading, math, and science that is known as the Nation's Report Card (<http://scim.ag/NAEP-2009>)

12 million The average extent, in square kilometers, of Arctic sea-ice cover during December 2010, according to the World Meteorological Organization. That makes it the lowest on record for December, about 1.35 million square kilometers below the 1979–2000 average.

12% The percentage of first-year U.S. college students who, when asked about seven common types of quantitative reasoning activities, said they had never done any of them, according to the 2010 National Survey of Student Engagement.

Frédéric Chopin's 'Madness' Diagnosed

In 1848, Polish composer and piano virtuoso Frédéric Chopin was performing at a house in Paris when he suddenly stopped in the middle of a piece and left the stage. He later wrote to a friend that he had seen creatures crawling out of his piano. Chopin is widely viewed as a tortured artist, but a new paper suggests his eccentricities might have been due to epilepsy.

Radiologist Manuel Vázquez Caruncho and neurologist Francisco Brañas Fernández of Xeral-Calde Hospital in Lugo, Spain, studied Chopin's writings and that of friends and pupils for descriptions of his hallucinations and wild behavior. Only a handful of neurological disorders produce the phantasmagoria that tormented Chopin, who didn't abuse drugs or alcohol. The authors rule out schizophrenia and other common psychoses because Chopin's hallucinations were visual,

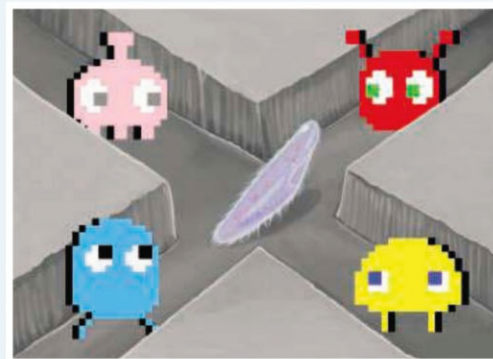
Random Sample

Petri Arcade

Long before he became a bio-engineer, Ingmar Riedel-Kruse was a typical 12-year-old video game aficionado. "I wasted a year of my life doing that—'wasted' in a good sense," he recalls. He soon moved on from playing with computers to programming them and, eventually, to biotechnology research. About a year ago, while reading a Wikipedia article about the history of video games, he had an inspira-

tion: Why not start gaming with actual, live microbes? After "lots of trial and error," Riedel-Kruse says, he and his colleagues at Stanford University developed a basic game console that nudges paramecia around a microfluidic chamber with chemical gradients or mild electric fields. A microscope camera pipes images of the wriggling protozoans into a laptop game window. Games include soccerlike "Ciliaball"; "Pac-mecium," in which paramecia gobble virtual yeast dots while avoiding a cartoon zebrafish larva; and "Pond Pong," in which two players bat the microbes back and forth by releasing chemicals from a needle tip. Descriptions of the whole microarcade, including games involving yeast and DNA, appear this month in the journal *Lab on a Chip*.

Riedel-Kruse says putting biotic games on the Internet could give researchers a way to crowdsource real-time biology experiments to online players. He's also working on bringing them into schools to inspire future scientists—after all, it worked for him.



not auditory, and because he lacked other telltale symptoms such as eye problems or migraines. His short hallucinatory episodes are a hallmark of temporal lobe epilepsy, the team reports online in *Medical Humanities*.

Other researchers call the proposal interesting but perhaps too subtle. The authors themselves admit it is difficult to be conclusive without the ability to observe Chopin himself. However, Caruncho points out, testimonies from witnesses are key in diagnosing epilepsy even today.

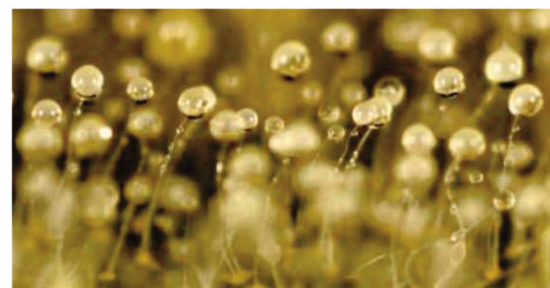
<http://scim.ag/mad-chopin>

The World's Smallest Farmers

It's too bad they don't make microscopic overalls. A study published online last week in *Nature* finds that the single-celled organism *Dictyostelium discoideum* harvests bacteria like farmers harvest crops.

An individual *D. discoideum*, or "Dicty," amoeba cell can live independently, slurping up bacteria in the soil. When the food is gone, it joins with its comrades to form a tiny sluglike organism that wriggles to greener pastures. Once there, the slug becomes a stalk with a fruiting body (pictured)—a tiny globe on top that releases spores, each spawning a single amoeba.

Debra Brock, a graduate student in ecology and evolutionary biology at Rice University in Houston, Texas, was studying spores from wild Dicty amoebae when she saw something she'd never seen before: bacteria in the fruiting body. To find out whether the bacteria were just an infection,



she gave the spores antibiotics, then placed them on a fresh patch of bacteria. The spores that had originally harbored bacteria picked up the bugs again, indicating that they were collecting bacteria. Other experiments showed that the amoebas "planted" their new environments with bacteria and harvested them. Several animals are known to farm; some ant species tend fungi, for example. But researchers say it's surprising to find the behavior in such a simple organism.

<http://scim.ag/tiny-farmers>



U.S. SCIENCE POLICY

Collins Sparks Furor With Proposed NIH Reshuffling

A plan to create a new center aimed at developing drugs at the U.S. National Institutes of Health (NIH) has many biomedical researchers in an uproar. Since NIH Director Francis Collins endorsed the plan in mid-December, it has drawn a flood of concerned and sometimes angry comments, mainly because creating a new NIH center would entail breaking up another center. Questions last week from a congressional committee have cast uncertainty over Collins's plan to launch the new center by next October.

Collins defended the proposal last week: "I will not be apologetic for wanting to see the scientific opportunities that relate to translation approached in a very bold way," he told *Science*. "And of course change is always distressing, especially if people aren't quite sure where it's going. So I understand the anxiety that currently exists."

The reorganization grew out of a 7 December 2010 recommendation by an NIH advisory board, called the Scientific Management Review Board (SMRB), to create a center aimed at speeding the development of biomedical discoveries into treatments (*Science*, 10 December 2010, p. 1462). Dubbed the National Center for Advancing Translational Sciences (NCATS), it would fold together \$632 million in NIH programs focused on clinical research and drug discovery and development that are now at other institutes. It would also house the Cures Acceleration Network, a drug development program created by

Congress last year that, if fully funded, would bring NCATS's budget up to \$1.1 billion.

The translational center has gotten a mixed response. Although some have questioned whether drug development fits NIH's mission, *The New York Times* gave it mostly positive coverage last week. Other worries, however, stem from NIH's decision to also disband its National Center for Research Resources. NCRR's largest program, the \$490 million Clinical and Translational Science Awards, would move into NCATS. NIH officials want to move the remaining 60% of NCRR, including grants for minority institutions and support for large shared instruments and animal models, to other institutes.

Since mid-December, this proposed reorganization has triggered more than 1200 comments on an NIH feedback site. Many scientists worry that splitting up NCRR components would be harmful—for example, putting the comparative medicine program's primate models in one institute and models such as zebrafish in another. Complaints continued last week when NIH Deputy Director Lawrence Tabak posted online a "straw model" showing how the pieces of NCRR would be distributed. Some have a designated home, but most have been assigned to an "interim infrastructure unit" in the NIH director's office—which, as some pointed out, looks much like NCRR. Collins says much of the interim unit would eventually go to the National Institute of General

Mover and shaker. NIH Director Francis Collins says he is "not apologetic" about a plan to dismantle one center to create a new one.

Medical Sciences (NIGMS), NIH's basic research institute.

Critics within and outside NIH are incensed by how decisions have been made. "The SMRB process simply backed into a foregone conclusion," and NCRR staff have received "an astounding level of ... disrespect," said one commenter on the feedback site. The feeling is that "Dr. Collins is accustomed to getting what he wants," says biochemist Mark Lively of Wake Forest University School of Medicine in North Carolina, who is a member of the NCRR advisory council.

In last week's interview with *Science*, Collins said statutory requirements compelled him to notify Congress of his plan to abolish NCRR before gathering public input. NIH's parent organization, the Department of Health and Human Services (HHS), sent those letters on 14 January. He said, "In my best of all worlds, I would have wanted ... a lot more discussion."

Collins also said he wasn't influenced by a law limiting NIH to no more than the current 27 institutes and centers. He could have asked Congress to allow NIH to exceed the cap temporarily until it merges its alcoholism and drug abuse institutes next year, but "I didn't even ask." Many NCRR programs fit more logically in other institutes, he says.

A task force co-chaired by Tabak plans to finish the plan for NCRR by March so it can be part of NIH's 2012 budget request. Meanwhile, Congress has 180 days to object to the reorganization. Last week, John Bartrum, a staffer on the House of Representatives appropriations subcommittee that oversees HHS's budget, sent HHS and NIH a long list of questions. Bartrum's e-mail says "we have not taken any position" on either NCATS or NCRR, but it asks for an explanation of NCATS's mission, details of its budget, and how NIH decided to eliminate NCRR.

One concern is that some of the programs NCATS would house are now funded by a pot of money in the NIH director's office for projects that are supposed to end after a few years. It's not clear how NCATS would maintain funding for them at a time when the overall NIH budget is expected to stay flat. But NIH officials said that contrary to a statement in *The New York Times* story, NIH has "no plans to 'cannibalize'" other parts of NIH to support NCATS.

—JOCELYN KAISER

CREDIT: GETTY IMAGES

HUMAN EVOLUTION

Did Modern Humans Travel Out of Africa Via Arabia?

JEBEL FAYA, UNITED ARAB EMIRATES—The barren desert and hills here seem wholly inhospitable, with sparse rain and sandy soil supporting only a few nomadic Bedouin. But things were different 125,000 years ago, when the desert was savanna, with plentiful water and game, and under the protection of a rock overhang, a group of hominids whiled away their time making stone tools. A German-led team argues on page 453 that these tools were made by modern humans who may have crossed directly from Africa as part of a migration spreading across Europe, Asia, and Australia. Although most researchers agree that our species came out of Africa in one or more waves (see p. 392), those dates are more than 50,000 years earlier than most believe our ancestors left the continent.

The audacious claim by Simon Armitage of Royal Holloway, University of London, and colleagues is sparking interest and controversy. “This is really quite spectacular,” says archaeologist Michael Petraglia of the University of Oxford in the United Kingdom, who has previously argued that *Homo sapiens* left Africa before the massive eruption of an Indonesian volcano 74,000 years ago, a catastrophe thought to have left much of Asia unlivable for early humans (*Science*, 5 March 2010, p. 1187). “It breaks the back of the current consensus view.” But others, such as archaeologist Paul Mellars of the University of Cambridge in the United Kingdom, say that although the discovery is important and well dated, the conclusions are flawed. “I’m totally unpersuaded,” he says. “There’s not a scrap of evidence here that these were made by modern humans, nor that they came from Africa.”

The debate centers on a collection of stone tools found here at Jebel Faya, a long limestone mountain an hour’s drive from the bustling urban center of Sharjah and 55 kilometers from the Persian Gulf. A rock shelter indents the mountain’s end, a few meters above a desolate plain where only camels graze today. The overhang is modest, but it has sheltered humans for millennia, say excavators Hans-Peter and Margarethe

Uerpmann of the University of Tübingen in Germany. They began digging here in 2003, uncovering artifacts from the Iron, Bronze, and Neolithic periods before hitting material from the Middle Paleolithic era, roughly 300,000 to 30,000 years ago. Using single-grain optically stimulated luminescence, which measures how much time has passed since materials were last exposed to light, the team dated the oldest set of artifacts, including stone hand axes, blades, and scrapers, to about 125,000 years ago.

Arabia and its fierce deserts have long been seen more as obstacles than conduits to human migration, and most archaeology here has focused on histori-

during this period in East Africa, when our own species was the only known hominin on that continent. Other hominins, such as the Neandertals who populated Europe and north Asia, did not use this combination of tools and were not likely to have been in Arabia, he says. That makes the African origin likely “by process of elimination.”

Marks says the tools don’t resemble those from Israel or the Aterian tools from the same era in North Africa (*Science*, 7 January, p. 20). He suggests that *H. sapiens* may have left Africa in different waves, with the Arabian tools representing a migration launched from East Africa.

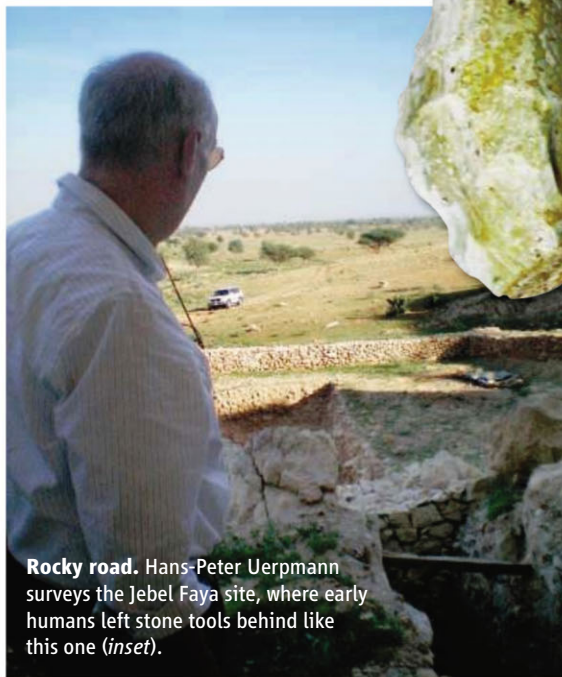
Petraglia agrees that it’s likely that *H. sapiens* made the tools and that they came from Africa. “This is out of the habitat range of Neandertals,” he notes. “So they make a really strong and plausible argument.” The team believes that these early modern humans may have even pushed on across the Persian Gulf, perhaps to India, Indonesia, and eventually Australia. Petraglia claims evidence of early *H. sapiens* in India both before and after the Indonesian eruption, though others dispute that assertion.

Mellars, in contrast, sees no evidence that the Jebel Faya artifacts are of an East African style. He says one of the bifacials is stout rather than narrow like those common in Africa and adds that the authors have not ruled out Neandertals and even *H. erectus* as the toolmakers. “Everything hinges on whether that material is explicitly African—and I don’t see that.”

Other researchers are enthusiastic about the Jebel Faya discovery but cautious about the conclusions. Archaeologist Mark Beech, a visiting fellow at the University of York in the United Kingdom who has worked extensively in the United Arab Emirates, praises the paper but adds: “One site does not confirm the out-of-Africa-via-Arabia hypothesis.”

Hans-Peter Uerpmann agrees, saying that fossil bones are needed “before we can be absolutely sure” that the tools were made by *H. sapiens*. Other researchers are hot on the trail: Petraglia leaves this month to continue work in Saudi Arabia, and other archaeologists plan to comb Arabian caves and sands for signs that our ancestors passed this way.

—ANDREW LAWLER



Rocky road. Hans-Peter Uerpmann surveys the Jebel Faya site, where early humans left stone tools behind like this one (inset).

cal times. Recent studies, however, show wetter periods such as one that began around 130,000 years ago. And a spate of findings in the past 25 years show that hominins were in the region during the Middle Paleolithic. Early *H. sapiens* skulls and tools from Skhul and Qafzeh caves in Israel are now dated to 100,000 to 130,000 years ago, for example.

Co-author Anthony Marks of Southern Methodist University in Dallas, Texas, says the combination of artifacts from Jebel Faya, such as two-sided blades and small hand axes, is remarkably similar to assemblages made



EPIDEMIOLOGY

Despite Sensitivities, Scientists Seek To Solve Haiti's Cholera Riddle

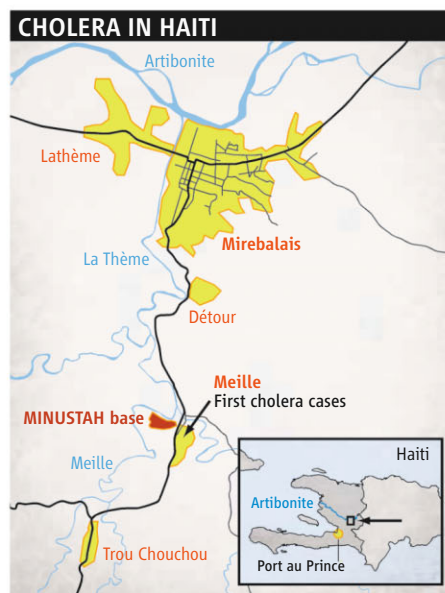
When French epidemiologist Renaud Piarroux came back from a 3-week mission to Haiti in late November, he faced a dilemma. Piarroux, who had been invited by the Haitian government to investigate the country's explosive cholera outbreak, was convinced that the bacterium had been introduced by Nepalese soldiers taking part in the United Nations Stabilization Mission in Haiti (MINUSTAH)—and he wanted to present his evidence. At the same time, he was leery of exacerbating tensions in Haiti, where angry mobs had already demanded the departure of the Nepalese. Even after his confidential report for the Haitian government was leaked to the press, Piarroux didn't talk to reporters until he got permission from French authorities. "I was very concerned," he says.

He's not alone. Several cholera experts told *Science* that nailing the source of the outbreak could potentially embarrass the United Nations, distract from the day-to-day fight to control the outbreak, and even lead to violence. So their passion for traditional shoe-leather epidemiology has been tempered by diplomatic and strategic concerns.

Indeed, prominent cholera scientists declined to discuss the issue with *Science* or would only speak off the record. The U.S. Centers for Disease Control and Prevention (CDC) in Atlanta is investigating the source, but a spokesperson referred questions about it to a panel charged by U.N. Secretary-General Ban Ki-moon with investigating the outbreak. (The panel's chair, Alejandro Cravioto of the

International Centre for Diarrhoeal Disease Research, Bangladesh, in Dhaka says none of its four members will speak to the press until their job is finished in April.) Meanwhile, a recent editorial in *The Lancet Infectious Diseases* cautioned against "apportioning blame" and declared the hunt for the source "a matter of scientific curiosity for the future."

Yet several scientists say that attitude is wrong-headed. Knowing how the outbreak started is very important, says Matthew



Ground zero? Because the first cholera cases occurred in Meille, a French epidemiologist thinks the Nepalese base is the source.

Pointing fingers. Haitian protesters have accused Nepalese peacekeepers of introducing cholera into the country.

Waldor of Harvard Medical School in Boston, because it could help prevent outbreaks from happening elsewhere. Besides, "if an epidemic killed 4000 people in Europe or the U.S., we would want to know exactly where it came from," says Piarroux. "So why not when the same happens in Haiti?"

Scientific results published in the past 2 months support an Asian origin for the outbreak but say nothing about Nepal. In November, CDC scientists reported in *Morbidity and Mortality Weekly Report* that pulsed-field gel electrophoresis—a widely used method to type microbial strains—showed that Haiti's cholera was indistinguishable from strains "found in countries in South Asia and elsewhere."

Sequencing the strain's entire genome and comparing it with that of other strains can provide more detailed information, and a first stab at that came on 6 January in a paper by Waldor and 15 other researchers in *The New England Journal of Medicine*. The group sequenced two samples from Haiti, two from Bangladesh collected during 2002 and 2008 outbreaks, and a Peruvian strain from 1991. They found that the Haitian strains were closely related to those from Bangladesh but not to the one from Peru.

Like CDC, the group is now sequencing a much larger number of samples, including one taken in Nepal about 5 years ago, says Waldor, who's also trying to get his hands on a sample from the outbreak that occurred in Nepal last fall, the same time the peacekeepers departed for Haiti. But whether additional samples will provide a definitive answer is not clear, he says. *Vibrio cholerae* doesn't evolve quickly, so there may not be enough differences between strains from different countries to tell them apart.

But for Piarroux, a researcher at the University of the Mediterranean in Marseille who previously investigated several African cholera outbreaks, there's enough circumstantial evidence to clinch the case. Working with epidemiologists of Haiti's Ministry of Public Health and Population, he found that the very first wave of cholera cases occurred in a village in central Haiti called Meille, where many inhabitants collected their drinking water from a stream, also named Meille, just downriver from the Nepalese MINUSTAH camp. During the first days of the epidemic, the Haitians noticed a pipe carrying a nauseating liquid from a septic tank inside the camp to the stream. The pipe—which was mentioned

CREDITS (TOP TO BOTTOM): REUTERS; ADAPTED FROM R. PIARROUX

in a Haitian report of which *Science* obtained a copy—was later removed, Piarroux says in his own report. (A paper he wrote about the outbreak is currently under review.)

Cholera can travel the world inside people's intestines, even unnoticed, because many infected people have few or no symptoms. But Piarroux does not believe that's what happened in Haiti. Based on the number of people who got sick in the first wave of the outbreak farther downriver, he calculated that the Nepalese must have had dozens of patients and dumped hundreds of liters of contaminated stool. As he wrote in his leaked report, there was a "massive contamination" of the Artibonite River that suggests a large outbreak inside the camp. A spokesperson for MINUSTAH did not respond to e-mailed questions, but the United Nations has denied that soldiers were sick.

Waldor says he finds Piarroux's evidence "suggestive but not absolutely conclusive." There are ways to collect more evidence—for instance, by testing the Nepalese soldiers for antibodies against *V. cholerae*. "It may be getting late for that," Waldor cautions, because antibodies peak after about a month.

Some scientists don't believe foreigners introduced cholera at all, despite the molecular clues. Most prominent among them is Rita Colwell, a veteran microbiologist at the University of Maryland, College Park. Colwell believes that most cholera outbreaks are caused by bacteria that lurk locally and proliferate when conditions are favorable—in this case, perhaps a climate event called La Niña. To Waldor, the idea that a microbe so closely resembling a South Asian strain would emerge in Haitian waters is "frankly absurd." (Colwell e-mailed *Science* that she was "in Bangladesh working on cholera and unable to respond" to questions.)

If the Nepalese introduced cholera, says Waldor, several measures could be considered to prevent a repeat. Aid workers or peacekeepers from cholera-endemic countries who are sent to cholera-free but vulnerable places like Haiti could be screened in advance, for instance, or given prophylactic antibiotics.

But Harvard cholera scientist Edward Ryan counters that testing thousands of soldiers using rectal swabs would be time-consuming and costly—and that testing isn't very accurate. Prescribing antibiotics would pose problems, such as adverse reactions and cause drug resistance in *V. cholerae* and other microbes. What's more, says CDC epidemiologist Eric Mintz, businesspeople, visiting relatives, and tourists would also have to be tested. "It wouldn't be very practical," Mintz says.

—MARTIN ENSERINK

ERADICATION

Pressure Growing to Set a Date to Destroy Remaining Smallpox Stocks

The fate of the world's last stocks of the deadly smallpox virus is once again being debated. Last week, the executive board of the World Health Organization (WHO) began discussing what research remains to be done with the live virus. In May, WHO's governing body, the World Health Assembly (WHA), will decide whether to set a firm deadline for the stock's destruction. Contrary to some media reports that the executive board recommended keeping the stocks, no conclusions were reached, says a WHO spokesperson.

Smallpox, or variola, killed hundreds of millions of people before it was declared eradicated in 1980. Only two labs still hold tightly

been made, they noted: 48 strains of the virus have been sequenced, and scientists have also developed new diagnostics, a safer version of the standard smallpox vaccine, new vaccines for immune-compromised people, and two new candidate antiviral drugs. But progress has been slow on developing a non-human primate model for smallpox infection, the panel said.

WHO then asked another advisory group of infectious-disease experts outside the pox-virus field to review the scientific report; they recommended a limited scope of research. Although research should continue on vaccines, live virus is needed only to test drugs in vitro. Nor is a nonhuman primate model worth further study, they said, suggesting that regulatory authorities base their decisions on animal studies of closely related viruses such as monkeypox and cowpox.

Both reports fed into a report from WHO's smallpox advisory committee that was formally presented at last week's meeting of the 34-member WHO Executive Board, where Russia, the United States, and—in a break with the past—some African countries reportedly expressed support for retaining stocks for research. But South Africa also urged that a firm date be set for destruction, sources told *Science*. There was no final agreement, says WHO spokesman Gregory Härtl; the "substantive discussion on the eventual destruction of smallpox stocks will take place in May at the World Health Assembly."

Tucker expects other countries to join the call for a destruction date. In May, the Third World Network (TWN), a Malaysian advocacy group, will also lobby for limiting the research allowed with live virus, as the non-poxvirus WHO advisory group suggested. That group "represents the broader public health view," says Edward Hammond, a consultant for TWN in Austin.

Tucker also supports destruction. But in a commentary this month in *Biosecurity and Bioterrorism*, he suggested a compromise to avoid a "diplomatic train wreck." The United States and Russia, which now hold 451 vials and 120 vials, respectively, should destroy all but 10 vials each. Countries should agree to make it a crime to synthesize the variola virus, and the United States and Russia should ensure that antiviral drugs and vaccines are readily available to developing countries.

—JOCELYN KAISER



Doomed? The United States and Russia insist live smallpox virus is still needed for research.

secured stocks of the virus: VECTOR near Novosibirsk, Russia, and the U.S. Centers for Disease Control and Prevention in Atlanta. A deadline for destroying these stocks first set by WHO in 1990 has been postponed several times after the United States and Russia argued that research should continue on vaccines, antiviral drugs, and diagnostics (*Science*, 25 January 2002, p. 598).

Since 2006, support has grown for destruction, says Jonathan Tucker, a bioweapons expert at Darmstadt University of Technology in Germany. Developing countries that had suffered severe outbreaks have argued that several research goals had already been achieved, and the others seem "unrealistic," says Tucker. In response, in 2007 WHA called for a review of research since 1999.

That panel of smallpox experts weighed in last October. Considerable progress has



PRIMATOLOGY

Last-Ditch Effort to Save Embattled Ape

BAWANGLING, CHINA—Dawn has broken over the rainforest, and time is of the essence. Wang Jin Qiang climbs the steep tropical terrain with the ease of a mountain goat, pausing only to let less fit companions catch their breath and tend to cuts and scratches from rattan thorns. After several bursts of brisk hiking, the ranger stops near a magnificent tulip oak with buttress roots that would turn a cathedral green with envy.

“Shhh!” he says, finger to lips.

A breeze has picked up. Almost lost amid the rustling of leaves is a melodious whoop. “That’s their morning call,” Wang whispers. We strain to listen for a minute, then Wang’s walkie-talkie crackles to life. It’s another ranger who has spent the past week camped out at a backcountry station solely to observe what may be the world’s most endangered primate, the Hainan gibbon. After some hushed chatter in local dialect, Wang says to us, “He spotted them. We’re close, let’s go.”

By the latest tally, there are only 22 Hainan gibbons—one family with 11 members, another with seven members, and four loners—remaining in their last refuge, Bawangling National Nature Reserve on southern China’s Hainan Island. Here, rangers and scientists hope to prevent the first primate extinction in recorded history. “This is the most likely primate to go extinct. We *have* to save it,” says Long Yongcheng, chief scientist for The Nature Conservancy’s China program and the go-to person for China’s primatology community.

Long and others are guardedly optimistic. Bawangling’s gibbon population dwindled to single digits a quarter-century ago, but

government protection and high fecundity have helped the species recapture some lost ground. Experts can only speculate whether the genetic bottleneck the gibbon is squeezing through will cripple its survival odds. “We’re down to a scary number,” says conservation biologist Chan Pui Lok Bosco, head of Kadoorie Conservation China (KCC), a nonprofit in Hong Kong. But other species under similar genetic duress have pulled through.

Half a century ago, an estimated 2000 gibbons roamed the rugged interior of Hainan, now known for its beach resorts. Villagers had avidly hunted the gibbons to use their body parts in traditional medicine. But the big blow came in the 1960s, when much of Hainan’s lowland rainforest—the gibbon’s preferred habitat—was converted to rubber plantations. The gibbons retreated to higher terrain, where subpopulations guttered and winked out. By the 1990s, a swath of land in Bawangling some 600 to 900 meters above sea level had become their last redoubt.

Realizing that something precious was about to be lost, provincial authorities in the 1990s banned logging in Bawangling and

Picking up the scent. Ranger Wang Jin Qiang listens for the gibbon’s morning call.

cracked down on poaching, which carries a 10-year prison sentence. By then most villagers had sworn off hunting. Years ago, a local man and his entire family died suddenly after he had shot a gibbon, Chan says. A fortunate consequence, he says, is that in the Bawangling area “hunting is taboo.”

A new push to save the gibbon began in 2003, when Hainan’s forestry department invited Kadoorie to conduct a gibbon census and devise a conservation action plan in Bawangling. Chan and his colleagues confirmed that there are only two families left and that they are confined to suboptimal habitat. With few gibbon food trees at higher elevations, the gibbons must roam far to forage. For that reason, Chan says, “they have the largest home range of any gibbon species in the world.” Hainan gibbons are arboreal creatures; none has been observed to come down to the ground. To not interfere with the gibbons’ diet, KCC trained locals to collect seeds of fallen fruit, such as the gibbon’s apparent favorite, *tao lan* (*Pouteria annamensis*). KCC has since planted more than 80,000 food trees in degraded lowland in Bawangling, says Chan. And starting in 2005, KCC has sponsored four pairs of rangers—two for each gibbon family—to spend 5-day shifts in the backcountry monitoring the rare ape.

After 5 years of intense study, the prognosis is uncertain. Researchers are collecting droppings and hair to probe the genetic bottleneck. On the plus side, three females are now pregnant, and the gibbons are managing to avoid human encounters.

The gibbons are clearly skilled at that. After hearing their faint morning chorus, it took only 10 minutes for Wang and company to reach the area where the other ranger had called in the sighting. By the time we arrived, the gibbons were gone. We followed through

a singular land: jewel orchids with exquisite leaves, the ground punctured by inch-wide holes made by forest crabs before hibernating, towering sugar palms, and a stand of trees clad with strange leaves that came to life—the beating wings of thousands of iridescent blue and black butterflies.

The gibbons, however, eluded us. That’s a survival skill. As long as they steer clear of their larger-brained cousins, continue to make babies, and have access to enough food, says Wang, “I think they have a chance.” —RICHARD STONE



Rare sighting. Hainan gibbons, with baby, in Bawangling reserve.

CREDITS (TOP TO BOTTOM): R. STONE/SCIENCE; CHEN QING/BAWANGLING NATIONAL NATURE RESERVE

CELL BIOLOGY

Telling Time Without Turning On Genes

Our planet's spinning on its axis has a profound effect on all living organisms. Creatures from bacteria to humans have internal timekeepers that keep them in sync with Earth's 24-hour day-and-night cycle. Researchers have identified a host of genes and proteins that help track time, controlling daily cycles of sleep and wakefulness, hunger, and metabolism in dozens of organisms and cell types. Most of the timekeepers discovered so far have depended on gene transcription, the process in which cells use the information stored in genes to make proteins, to drive the complex feedback loops of molecules that make up a cell's internal clock.

Now researchers have found evidence—in people and in algae—for a eukaryotic circadian clock that works independently of gene activity. The clock is present in human red blood cells, which lack a nucleus and so don't make any new proteins, and a eukaryotic alga called *Ostreococcus tauri*. This protein-based timekeeper, says Andrew Millar, a chronobiologist at the University of Edinburgh in the United Kingdom and an author on one of the two studies, might represent an evolutionarily ancient way of keeping cellular time.

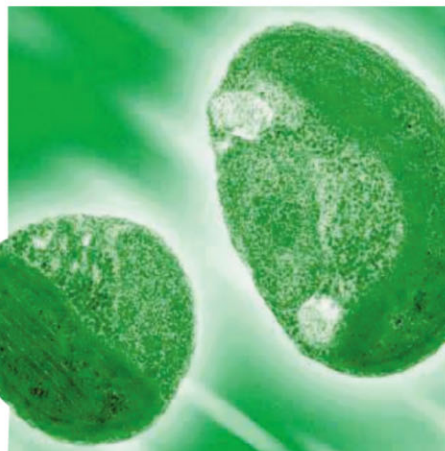
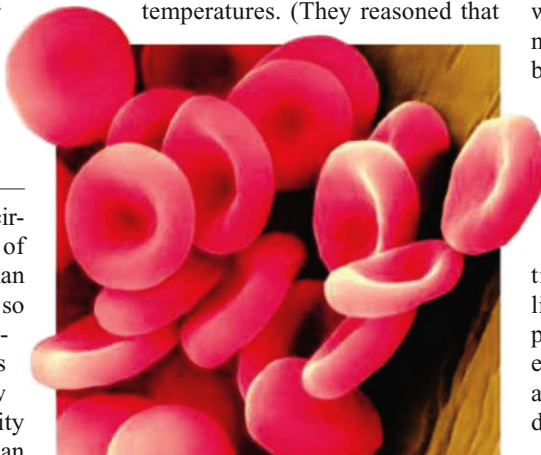
There had been previous evidence that some circadian clocks could run independently of gene transcription. In 2005, researchers showed that the clock proteins from cyanobacteria could run on their own without input from the cell's nucleus (*Science*, 15 April 2005, p. 414). But the new papers, published this week in *Nature*, are the first to demonstrate a transcription-free clock in eukaryotic cells.

Other work had hinted that eukaryotes might not always rely on transcription for circadian timing, but it had been difficult to prove the case because chemicals that block protein synthesis have many side effects on cells. John O'Neill and Akhilesh Reddy, both neuroscientists at the University of Cambridge in the United Kingdom, wondered whether they could use red blood cells as a naturally occurring example of a transcription-free cell.

Reddy had shown in previous studies that a protein called peroxiredoxin (PRX), an antioxidant that helps to protect cells against free radicals, was involved in circadian cycles in liver cells. So he and O'Neill isolated red blood cells from three healthy volunteers, kept the cells at a constant temperature and in complete darkness for 60 hours, and then

took samples of the cells and measured the oxidation status of PRX every 4 hours. Sure enough, the proteins showed a roughly 24-hour cycle of oxidation.

The ability to react to environmental stimuli is a key characteristic of circadian clocks, so the researchers tested whether they could "entrain" the red blood cells by exposing them to 12-hour cycles of higher and lower temperatures. (They reasoned that



Ancient timekeeper? Human red blood cells (top) and the alga *Ostreococcus* have a similar circadian clock.

red blood cells might react to the body's daily variations in temperature, which is highest in the evening and lowest in the morning.) The PRX rhythms responded to the temperature cues, suggesting that this clock could react to environmental signals.

Before moving to Cambridge, O'Neill had worked in Millar's lab with *Ostreococcus*, looking for rhythm keepers in the cells that worked independently of transcription. The alga has a characteristic that made it especially useful for the studies: After several days

in constant darkness, it shuts down its protein-making machinery. However, when the lights are turned back on, the cells show evidence that they are still keeping time, even in the absence of new proteins. No one had pinned down the molecules involved in this alga's mystery clock.

The researchers decided to check whether the alga's PRX protein, which is very similar to the human one, undergoes a daily cycle like the one in red blood cells. They found that under normal 12-hour dark-light cycles, the oxidation of the PRX protein rose and fell in a 24-hour pattern. The pattern was still evident when the algae were kept in constant darkness, even when chemicals were added that block protein manufacture.

"We couldn't believe it. We did [the experiments] again and checked" whether they might have made some mistake, Reddy says. The similarity in two such different organisms was a surprise because so far the clock genes identified in unrelated organisms have very little in common with each other—those in plants, for example, are completely different from those in animals. "No one has been able to show such a similarity across such diverse organisms," Reddy says.

It is not yet clear whether the PRX proteins are a "hand" on the cellular clock—a read-out—or whether they are part of the machinery that actually helps the cells keep time. And no one is suggesting that the transcription-based clock is unimportant in human, or even alga, cells; the researchers are working to find out exactly how the two clocks complement or back each other up. Joseph Bass, a chronobiologist at Northwestern University in Evanston, Illinois, notes that the PRX proteins play a role in cell metabolism and energy use, which fits in with recent studies in humans that have found strong links between disrupted circadian patterns and metabolic disorders.

The PRX proteins will give scientists a new tool to probe the workings of circadian clocks across a range of organisms, says Martha Merrow, a chronobiologist at the University of Groningen in the Netherlands. And Reddy suggests that the new work may help scientists finally pin down the circadian mechanism in organisms, such as yeast and *Caenorhabditis elegans*, for which no clock-related genes have been identified. "Because bacteria, plants, and humans have these completely nonoverlapping [clock] genes, people have assumed that clocks had evolved completely independently," Reddy says. "They might all be linked through a common mechanism after all."

—GRETCHEN VOGEL

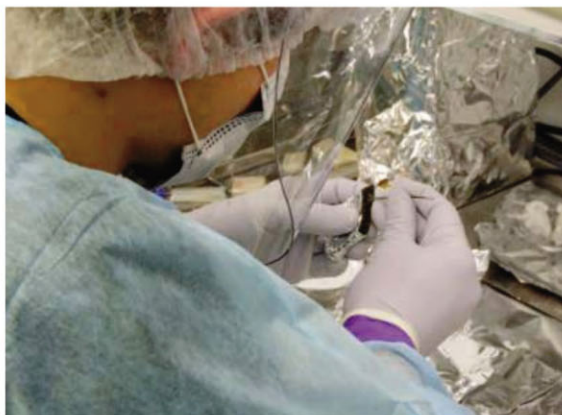


A New View Of the Birth of *Homo sapiens*

New genomic data are settling an old argument about how our species evolved

FOR 27 YEARS, CHRIS STRINGER AND Milford Wolpoff have been at odds about where and how our species was born. Stringer, a paleoanthropologist at the Natural History Museum in London, held that modern humans came out of Africa, spread around the world, and replaced, rather than mated with, the archaic humans they met. But Wolpoff, of the University of Michigan, Ann Arbor, argued that a single, worldwide species of human, including archaic forms outside of Africa, met, mingled and had offspring, and so produced *Homo sapiens*. The battle has been long and bitter: When reviewing a manuscript in the 1980s, Wolpoff scribbled “Stringer’s desperate argument” under a chart; in a 1996 book, Stringer wrote that “attention to inconvenient details has never been part of the Wolpoff style.” At one tense meeting, the pair presented opposing views in rival sessions on the same day—and Wolpoff didn’t invite Stringer to the meeting’s press conference. “It was difficult for a long time,” recalls Stringer.

Then, in the past year, geneticists announced the nearly complete nuclear genomes of two different archaic humans: Neandertals, and their enigmatic eastern cousins from southern Siberia. These data provide a much higher resolution view of our past, much as a new telescope allows astronomers to see farther back in time in the universe. When compared with the genomes of living people, the ancient genomes allow anthropologists to thoroughly test the competing models of human origins for the first time.



Going back in time. A researcher extracts DNA from a fossil.

The DNA data suggest not one but at least two instances of interbreeding between archaic and modern humans, raising the question of whether *H. sapiens* at that point was a distinct species (see sidebar, p. 394). And so they appear to refute the complete replacement aspect of the Out of Africa model. “[Modern humans] are certainly coming out of Africa, but we’re finding evidence of low levels of admixture wherever you look,” says evolutionary geneticist Michael Hammer of the University of Arizona in Tucson. Stringer admits: “The story has undoubtedly got a whole lot more complicated.”

But the genomic data don’t prove the classic multiregionalism model correct either. They suggest only a small amount of interbreeding, presumably at the margins where invading moderns met archaic groups that were the worldwide descendants of *H. erectus*, the human ancestor that left Africa 1.8 million years ago. “I have lately taken to talking about the best model as replacement with hybridization, ... [or] ‘leaky replacement,’ ” says paleogeneticist Svante Pääbo of the Max Planck Institute for Evolutionary Anthropology in Leipzig, lead author of the two nuclear genome studies.

The new picture most resembles so-called

CREDITS: MAX PLANCK INSTITUTE FOR EVOLUTIONARY ANTHROPOLOGY

Ancient abode. A finger and molar (*inset*) of a new type of human were found in Denisova Cave, Siberia.

assimilation models, which got relatively little attention over the years. “This means so much,” says Fred Smith of Illinois State University in Normal, who proposed such a model. “I just thought ‘Hallelujah! No matter what anybody else says, I was as close to correct as anybody.’”

Evolving models

Stringer and others first proposed Africa as the birthplace of modern humans back in the mid-1980s. The same year, researchers published a landmark study that traced the maternally inherited mitochondrial DNA (mtDNA) of all living people to a female ancestor that lived in Africa about 200,000 years ago, dubbed mitochondrial Eve. She caught the attention of the popular press, landing on the cover of *Newsweek* and *Time*.

Additional studies of living people—from Y chromosomes to snippets of nuclear DNA to the entire mtDNA genome—consistently found that Africans were the most diverse genetically. This suggests that modern humans arose in Africa, where they had more time to accumulate mutations than on other continents (*Science*, 17 November 2006, p. 1068). Meanwhile, ancient DNA technology also took off. Pääbo’s group sequenced first a few bits of Neandertal mitochondrial DNA in 1997, then the entire mitochondrial genomes of several Neandertals—and found them to be distinct from those of living people. So ancient DNA, too, argued against the idea of mixing between Neandertals and moderns. Over the years the replacement model became the leading theory, with only a stubborn few, including Wolpoff, holding to multiregionalism.

Yet there were a few dissenting notes. A few studies of individual genes found evidence of migration from Asia into Africa, rather than vice versa. Population geneticists warned that complete replacement was unlikely, given the distribution of alleles in living humans. And a few paleoanthropologists proposed middle-of-the-road models. Smith, a former student of Wolpoff’s, suggested that most of our ancestors arose in Africa but interbred with local populations as they spread out around the globe, with archaic people contributing to

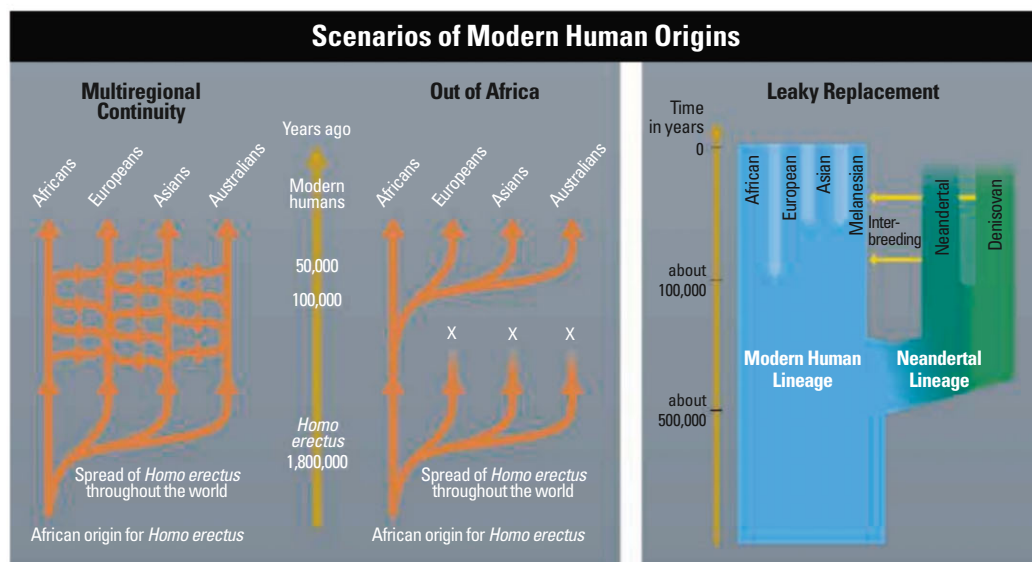
about 10% of living people’s genomes. At the University of Hamburg in Germany, Gunter Brauer similarly proposed replacement with hybridization, but with a trivial amount of interbreeding. But neither model got much traction; they were either ignored or lumped in with multiregionalism. “Assimilation got kicked so much,” recalls Smith.

Over time, the two more extreme models moved toward the middle, with most multiregionalists recognizing that the chief ancestors of modern humans arose in Africa. “The broad line of evolution is pretty clear: Our ancestors came out of Africa,” says biological anthropologist John Relethford of the State University of New York College at Oneonta. “But what happens next is kind of complex.”

the genome was neither a Neandertal’s nor a modern human’s, yet the girl was alive at the same time, dating to at least 30,000 years ago and probably older than 50,000 years. Her DNA was most like a Neandertal’s, but her people were a distinct group that had long been separated from Neandertals.

By comparing parts of the Denisovan genome directly with the same segments of DNA in 53 populations of living people, the team found that the Denisovans shared 4% to 6% of their DNA with Melanesians from Papua New Guinea and the Bougainville Islands. Those segments were not found in Neandertals or other living humans.

The most likely scenario for how all this happened is that after Neandertal and Denisovan populations split about 200,000 years ago,



Changing views. Two models of modern human origins (*left*) are being challenged by new insights based on ancient DNA (*right*), which suggest some limited interbreeding between modern and archaic populations.

Genes from the past

Then in May 2010 came the Neandertals’ complete nuclear genome, sequenced from the bones of three female Neandertals who lived in Croatia more than 38,000 years ago. Pääbo’s international team found that a small amount—1% to 4%—of the nuclear DNA of Europeans and Asians, but not of Africans, can be traced to Neandertals. The most likely model to explain this, Pääbo says, was that early modern humans arose in Africa but interbred with Neandertals in the Middle East or Arabia before spreading into Asia and Europe, about 50,000 to 80,000 years ago (*Science*, 7 May 2010, pp. 680, 710).

Seven months later, on 23 December, the team published in *Nature* the complete nuclear genome of a girl’s pinky finger from Denisova Cave in the Altai Mountains of southern Siberia. To their surprise,

modern humans interbred with Neandertals as they left Africa in the past 100,000 years. Thus Neandertals left their mark in the genomes of living Asians and Europeans, says co-author Montgomery Slatkin, a population geneticist at the University of California, Berkeley. Later, a subset of this group of moderns—who carried some Neandertal DNA—headed east toward Melanesia and interbred with the Denisovans in Asia on the way. As a result, Melanesians inherited DNA from both Neandertals and Denisovans, with as much as 8% of their DNA coming from archaic people, says co-author David Reich, a population geneticist at Harvard Medical School in Boston.

This means *H. sapiens* mixed it up with at least two different archaic peoples, in at least two distinct times and places. To some, that’s starting to sound a lot like multiregionalism. “It’s hard to explain how good I feel

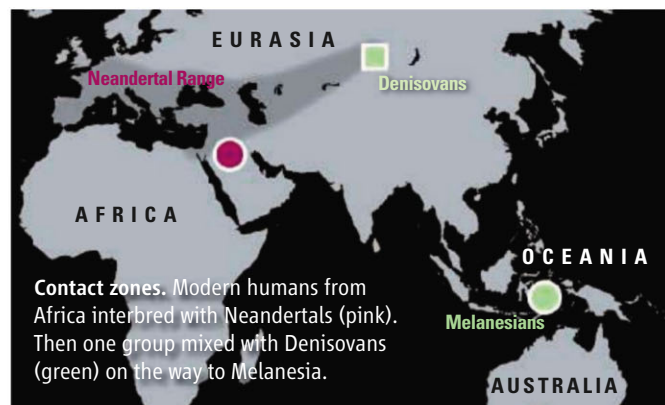
about this,” says Wolpoff, who says that seeing complete replacement falsified twice in 1 year was beyond his wildest expectations. “It was a good year.”

And yet the interbreeding with archaic humans seems limited—from 1% to 8% of some living people’s genomes. Stringer and many others don’t consider it full-scale multi-regional continuity. “I think interbreeding was at a low level,” says Slatkin, who says that if there had been a great deal of admixture, the genetic data would have revealed it already. Low levels of interbreeding suggest that either archaic people mated with moderns only rarely—or their hybrid offspring had low fitness and so produced few viable offspring, says population geneticist Laurent Excoffier of the University of Bern in Switzerland.

In any case, Reich notes that at least 90% of our genomes are inherited from Afri-

can ancestors who replaced the archaic people on other continents but hybridized with them around the margins. And that scenario most closely backs the assimilation models proposed by Smith and Brauer.

Of course, it’s possible that future data will overturn today’s “leaky replacement” model. Slatkin says he cannot rule out an alternative explanation for the data: The “archaic” DNA thought to have come from mating with Neandertals could instead stem from a very ancient ancestor that we shared with Neandertals. Most modern humans retained those archaic sequences, but Africans lost them. But



Slatkin says this “doesn’t seem very plausible,” because it requires modern human populations with the archaic DNA and those without it to have been partially isolated from each other in Africa for hundreds of thousands of years. And it seems even less probable that Melanesians and Denisovans are the only groups that retained a second set of archaic DNA motifs from a common ancestor shared by all modern humans, Neandertals and Denisovans. If those explanations do prove true, replacement would not be falsified.

In the wake of the big genome studies, other researchers such as Hammer are scrutinizing DNA from more living humans to further test the model. Researchers are also trying to pinpoint when admixture happened, which has significant consequences. At just what point did we evolve from archaic humans to become “modern” humans? “There are still archaic [genetic] features floating around until amazingly recently, until 40,000 years ago,” says Hammer. He wonders whether the process of becoming modern took longer and was more complex than once thought. “There’s no line you can draw and say everything after this is modern. That’s the elephant in the room.”

Meanwhile, paleoanthropologists are searching for fossils in Asia that might belong to the enigmatic Denisovan population—and might yield more ancient DNA. Paleoanthropologist Russell Ciochon of the University of Iowa in Iowa City and Wolpoff say there are several known, ambiguous fossils in Asia that might be candidates for early Denisovans. “I believe things were going on in Asia that we just don’t know about,” says Ciochon. “Before this paper on the Denisovans, we didn’t have any insight into this. Now, with this nuclear genome, I find myself talking about ‘the Denisovans.’ It’s already had an impact.”

As for Stringer and Wolpoff, both now in their 60s, their battle has mellowed. Their views, while still distinct, have converged somewhat, and they shared a beer at a Neandertal meeting last year. “The reason we get on well now,” says Stringer, “is we both think we’ve been proved right.”

—ANN GIBBONS

The Species Problem

Our ancestors had sex with at least two kinds of archaic humans at two different times and places—and those liaisons produced surviving children, according to the latest ancient DNA research (see main text, p. 392). But were the participants in these prehistoric encounters members of separate species? Doesn’t a species, by definition, breed only with others of that species?

These are the questions paleogeneticist Svante Pääbo dodged twice last year. His team published two papers proposing that both Neandertals and mysterious humans from Denisova Cave in Siberia interbred with ancient modern humans. But the researchers avoided the thorny question of species designation and simply referred to Neandertals, Denisovans, and modern humans as “populations.” “I think discussion of what is a species and what is a subspecies is a sterile academic endeavor,” says Pääbo, who works at the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany.

The question of how to define a species has divided researchers for centuries. Darwin’s words in *On the Origin of Species* still hold: “No one definition has satisfied all naturalists.” However, many scientists use the biological species concept proposed by Ernst Mayr: “groups of actually or potentially interbreeding natural populations, which are reproductively isolated from other such groups.”

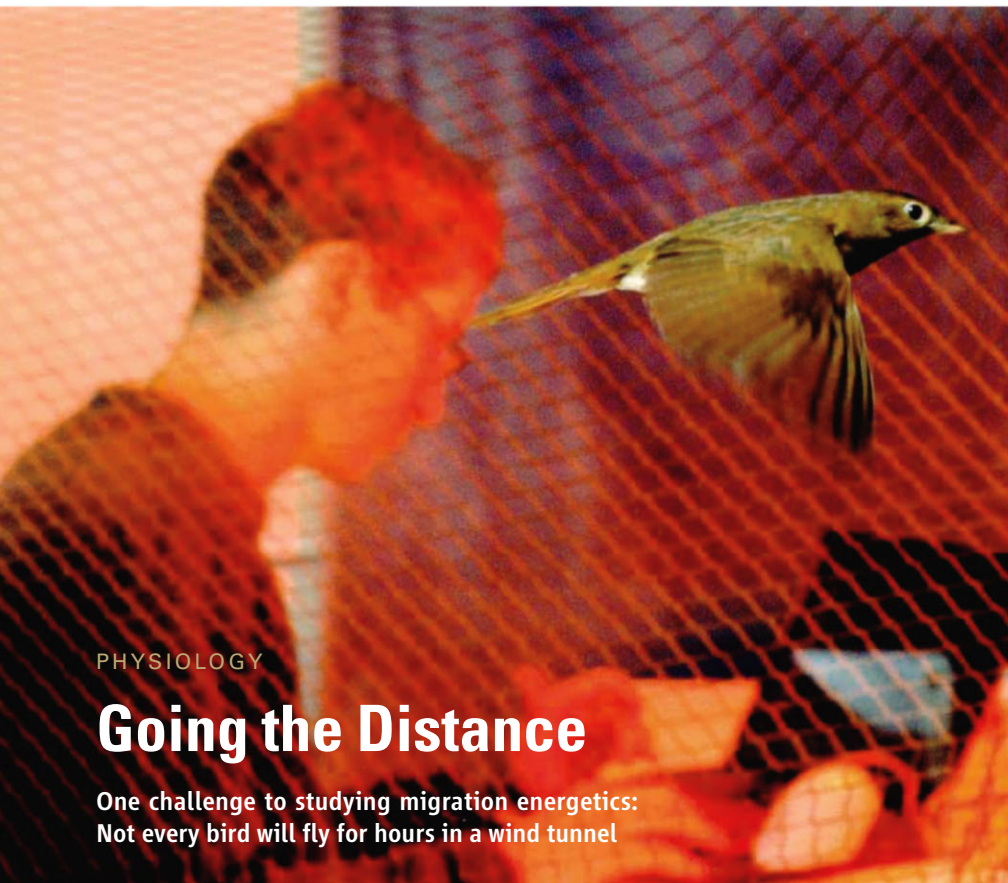
The draft versions of the Neandertal and Denisovan nuclear genomes show low levels of interbreeding between each of them and modern humans. Apply Mayr’s definition strictly, and all three must be considered *Homo sapiens*. “They mated with each other. We’ll call them the same species,” says molecular anthropologist John Hawks of the University of Wisconsin, Madison.

But that’s a minority view among paleoanthropologists. Many consider Neandertals a species separate from modern humans because the anatomical and developmental differences are “an order of magnitude higher than anything we can observe between extant human populations,” says Jean-Jacques Hublin, a co-author of Pääbo’s at Max Planck. In the real world, he says, Mayr’s concept doesn’t hold up: “There are about 330 closely related species of mammals that interbreed, and at least a third of them can produce fertile hybrids.”

There’s also no agreed-upon yardstick for how much morphologic or genetic difference separates species. That’s why Pääbo’s team avoided the species question a second time with respect to the Denisovans. These hominins are known only from a scrap of bone, a single tooth, and their DNA. They are genetically closest to Neandertals. The genetic distance between Denisovans and Neandertals, in fact, is only 9% larger than that between a living Frenchman and a living San Bushman in Africa, both of whom belong to *H. sapiens*. But so far Neandertals seem to have low genetic diversity, based on the DNA of six Neandertals from Russia to Spain. To Pääbo’s team, that makes the difference from the Denisovans significant.

Also, the Denisovan tooth doesn’t look much like that of a Neandertal. So the team considers them a distinct population but declined to name a new species. “Why take a stand on it when it will only lead to discussions and no one will have the final word?” asks Pääbo.

—A.G.



PHYSIOLOGY

Going the Distance

One challenge to studying migration energetics:
Not every bird will fly for hours in a wind tunnel

LONDON, CANADA—In a dimly lit room, Chris Guglielmo reaches into a cloth bag and gently pulls out a tiny bird. He checks that its feathers and wings are intact, then puts his hand inside a Plexiglas tunnel and releases it. All eyes in the room are mesmerized by the blur of beating wings as this yellow-rumped warbler flits about the chamber. An 8-meter-per-second headwind keeps the bird in place as it begins what Guglielmo hopes will be a multihour flight.

Moments later, the warbler perches on a net at the upper end of the tunnel. It can be coaxed back into the air, but it's clear that it prefers to stay put. "I'm a little bummed, but it's how it goes," says Guglielmo's graduate student, Alex Gerson, as he recaptures the bird.

A \$9.3 million grant has bought Guglielmo's group at the University of Western Ontario here a state-of-the-art bird research facility, complete with aviaries, surgery room, a bird-sized magnetic resonance imaging (MRI) machine, and a \$1.5 million wind tunnel, the only one in the world in which temperature, humidity, and barometric pressure can be controlled. A year old, the tunnel presents researchers with an unprecedented opportunity to probe the mysteries of migration in exquisite detail.

Guglielmo and Gerson want to understand fuel and water use by migrants. In the field, they follow birds that stop over to stock up during the fall and spring migration. What the birds eat can affect their ability to reach their final destination, and conservationists are keen to know which diets work best—and possibly provide them. "It's not enough, if you care about wild birds, to go out and count them and study their habitat," says Guglielmo. "You must know more about the mechanisms," and that's where the wind tunnel comes in. "It's providing us with a tool to answer questions that we could only answer indirectly [before]," says Scott McWilliams, a physiological ecologist at the University of Rhode Island, Providence.

Wind tunnels provide a controlled environment in which researchers can take before-and-after physiological measurements to assess the toll flight takes on fat stores, protein mass, water, and even immunological function when birds fly long distances. In addition, high-speed cameras and techniques for visualizing air flow enable Guglielmo's colleagues to assess the biomechanics of flapping wings, giving a better handle on the link between aerodynamics and migration.

Wind tunnel champ. Marcel Klaassen watches Blue, a bird that flew 16 hours and went nowhere.

But money can't buy birds that are keen to fly, and Guglielmo and his team have had mixed success finding willing avian partners. An early project using starlings did well, despite taking place when the tunnel was not quite finished. It netted "Super," a bird that always cooperates and will even fly into the wind tunnel on its own accord. But a study involving robins took months to identify five somewhat cooperative fliers; switching to Swainson's thrushes worked better. One immunological project involving a shorebird called a ruff is stranded because the birds show no inclination to take to the air. And Guglielmo has just started testing warblers to see if high-protein or high-carbohydrate diets make a difference in energy use during flight. "There's a lot of trial and error on what species of birds to use and how to find individuals that will fly," says Guglielmo.

Eat, drink, and fly

When Guglielmo gets discouraged, he thinks about Blue, a wind-tunnel record holder. In the mid-1990s, field ecologists Marcel Klaassen and Åke Lindström wanted to learn more about how stopovers influence subsequent migration. "In the wild, you can study a bird only a few days and then it's gone, literally gone," explains Lindström, based at Lund University in Sweden. Until that time, researchers had only tested non-migratory birds, such as homing pigeons and small parrots, in short wind-tunnel flights, "so we didn't know if what we found out was relevant" to migratory birds, he says. Lund had a newly built wind tunnel, one that later served as inspiration for Guglielmo's.

On the advice of a wind-tunnel expert, Lindström and Klaassen were trying to train thrush nightingales to fly from a stick for a food reward, but the birds were not cooperating. Getting one to stay in the air for even just a minute "was cause for celebration," Lindström recalls. That all changed when someone suggested removing the stick.

Blue looked back for his perch, wavered a little, then settled down to flying. In one trial, Blue kept going for 16 hours, showing no signs of slowing down even when the researchers decided it was time to go home to bed. Over the next 2 months, Blue made seven 12-hour flights, yielding data that led to five research papers on fuel use.

Online

sciencemag.org

S Podcast interview
with author
Elizabeth Pennisi.

Treading Air

Wind tunnels have come a long way since former DuPont president and amateur ornithologist Crawford Greenewalt built one of the first 50 years ago to study hummingbirds. A fan at the end of his 46-centimeter “tunnel” kept hummingbirds stationary while he filmed them flying; those high-speed images impressed amateurs and academics alike.

In 1994, Swedish biologists greatly improved on the concept, borrowing from designs used in engineering. Air was recirculated through a 20-meter-long rectangular chamber. Just upstream of the 2-meter-long test section, the tunnel widens and then narrows, which greatly reduces turbulence and makes it easier to assess how power requirements for flight vary with speed. In this setting, Anders Hedenström of Lund University in Sweden and his colleagues adopted an engineering technology that uses fog and lasers to visualize airflow. They documented for the first time what happens to air as it moves across a real bird’s wing. The tunnel can be tilted to allow birds to glide or “climb” to higher alti-

tudes. Over the years Hedenström and his colleagues have studied flight in 15 species, as well as in bats.

A copy of the Lund tunnel was built at the Max Planck Institute for Ornithology in Seewiesen, Germany, in 1999. There, 150 birds from 25 species have been evaluated.

When Chris Guglielmo started at the University of Western Ontario in London, Canada, in 2005, he realized those tunnels were too far away for him to include wind-tunnel studies routinely in his research. Others on campus wanted better bird-care facilities for their research, so he teamed up with nine colleagues and proposed the Advanced Facility for Avian Research. Much to their university president’s surprise, the Canada Foundation for Innovation approved this grant application in 2007.

The wind tunnel is the centerpiece of a new building that includes indoor and outdoor aviaries, some of which have small pools of water for shorebirds and waterfowl, acoustic chambers, behavior-observation rooms, and environmental chambers for controlling light cycles, as well as temperature and humidity. Made of steel, two stories high and 12 meters

long, the tunnel looks quite imposing. Birds fly in a 1-meter-high by 1.5-meter-wide octagonal Plexiglas cylinder, almost completely covered with blankets to discourage the birds from getting too close to the walls. Pressure, temperature, and humidity in the tunnel can all be modified to simulate different conditions, including high altitude.

The whole flight section is encased in a steel room, with air locks. Once the room and wind tunnel are locked down, “in a few minutes, you can go up to over 23,000 feet,” says Guglielmo. “There’s a lot of theory about what happens when birds fly at [high] altitudes, but nowhere in the world had we had a place to test that empirically.”

Guglielmo and his colleagues are still developing protocols for low-pressure research, but they have already simulated weather fronts. Scott MacDougall-Shackleton, also of Western Ontario, and Guglielmo put the birds into the tunnel at night and just before the lights go on, they cause a drop or rise in air pressure and temperature and observe how that affects the birds’ behavior when they wake up. It seems that when the birds sense bad weather coming on, they are much quicker to start feeding, says Guglielmo.

The tunnel also doubles as a studio for understanding the mechanics of bird flight. Working with Roi Gurka of Ben Gurion University in Beer-Sheva, Israel, Gregory Kopp and graduate student Adam Kirchhefer of Western Ontario have filled the tunnel with oil droplets and set up a laser field in front of a high-speed video camera that captures the movement of the droplets—and the air—as a bird flies. A second camera is trained on the bird itself. In particular, they are studying the movement of air at the wing tips. For these studies, a particularly cooperative starling named Super wears an orange-tinted mask to protect its eyes from the laser. “A few good seconds of video will keep you going for a long time,” says Guglielmo. And a sample size of one or two is sufficient. “It’s a different ball game when you want to look at migration,” where one needs multiple birds willing to fly for hours at a time.

—E.P.



Migration test bed. Researchers can control this wind tunnel’s temperature, humidity, and air pressure.

The work showed that the estimated power requirements for migratory flight were less than those for nonmigratory flights and suggested that birds burn protein as well as fat on their long journeys.

Searching for eager fliers like Blue, Guglielmo knew that the wind tunnel itself was not the problem. Already, McWilliams had had a successful run for his experiment on the effects of fatty acid composition and dietary oxidants on exercise performance,

even as the tunnel was being finished. In the summer of 2009, McWilliams’s team captured 120 starlings, feeding some of them food rich in olive oil (a monounsaturated fat) and others a diet high in canola oil (a polyunsaturated fat). His earlier experiments showed that migrating birds will choose fruits, such as viburnum or bayberry, that are high in polyunsaturated fats, even though metabolizing polyunsaturated fats generates more oxidative stress than

burning monounsaturated fats. McWilliams wanted to determine whether birds eating the canola oil diet flew more efficiently, burning less fat per hour, than birds eating olive oil, making the extra stress worth it.

The researchers look at how birds fuel their flights by determining the change in the ratio of fat to lean mass, and the rate at which the birds expend energy. Researchers have traditionally assessed fat content by checking the color and condition of

CREDIT: SCOTT MACDOUGALL-SHACKLETON

Taking measure. Briefly immobilized, this bird undergoes a magnetic resonance scan of its body composition.



the belly. A more accurate measurement using x-rays requires the birds to be anesthetized. The Western Ontario lab has a far more convenient tool, however: a custom-built quantitative magnetic resonance scanner that can produce an accurate readout of the lean mass, fat mass, and body water content quickly and noninvasively. It takes just 2 minutes, with the bird temporarily immobilized in a Plexiglas tube. “It’s no more difficult than weighing a bird,” says Guglielmo. “It’s really a new way to do these experiments.”

To assess how much energy the birds expend, the researchers inject birds just prior to flight with water containing heavier isotopes of hydrogen and oxygen. They then take blood samples before and after flight and use the change in the relative amounts of these stable isotopes to estimate carbon dioxide production, a proxy for energy expenditure.

McWilliams was eager to begin testing the effects of the different diets on flight energetics, but construction had fallen behind schedule, and the experiment was delayed for weeks. By September 2009, he couldn’t wait any longer. “It was a little touch and go,” he recalls. In between cycles of construction, he and his colleagues trained birds to fly in the wind tunnel and then, for the experiment, let each bird fly as long as it wanted, hoping for hourlong or longer flights. McWilliams is still analyzing the data, but he says The Nature Conservancy is eager to know the answer so it can better manage the preserves used by migratory birds.

Hit or miss

Guglielmo and Gerson knew from experience that finding the right species for their experiments, and the right individuals, was hit or miss. Gerson is tackling another aspect of flight energetics: water balance. Studies of birds that fly across deserts indicate that to make up for water loss, the birds break down their organs, harvesting water from the metabolism of proteins in muscles or the gut, for example. Gerson has been testing this idea by examining the change in body water, fat mass, and lean mass in birds flying in the wind tunnel.

American robins, common migrants of optimal size, seemed ideal. “But they didn’t work out so well,” says Guglielmo. Still, he and Gerson did learn something from robins: Hand-raised birds were no more likely to cooperate than wild-caught birds that were trained. So Gerson started trying whatever birds he could get his hands on, red-eyed vireos, gray catbirds, yellow-rumped warblers. Their conclusion: “Fat birds and calm birds fly,” quips Guglielmo.

Swainson’s thrushes seemed to work well. One went more than 5 hours nonstop, the equivalent of about 180 kilometers, using about 0.25 grams of fat per hour. Gerson has been testing them in 10% and 80% humidity, checking weight loss and using the MRI scanner to measure changes in water content and lean and fat mass before and after the flights. Preliminary results show that “if the bird flies in dry conditions, it will burn more of its lean mass to liberate more water,” he says.

Fat and calm is no guarantee of success, however. Silke Nebel, a postdoc, wants to

check how the state of the immune system affects flight. There is some evidence that birds with infections don’t fly as well, and it is important to quantify the handicap, particularly for epidemiologists trying to predict how far avian-borne diseases will spread. The tradeoff that may exist between immunocompetence and flight performance “is very difficult to study in a natural setting,” she points out.

The wind tunnel is too small for flying ducks and geese, so Nebel wants to use ruffs as stand-ins. However, the ruffs, which come from a captive colony, showed no inclination to migrate, despite having put on weight and being used to captivity. Nebel has tried hatching, raising, and training young. Even so, at 2 months old, these youngsters fly no more than a few minutes at a time. Her last hope is that as they mature, the birds will develop larger flight muscles that will let them stay in the air longer.

Flying without a net

Guglielmo settled on the yellow-rumped warbler because it is small, and the species is known to adapt well to captivity. The yellow-rumped warbler varies its diet seasonally, feasting on insects in the spring but turning more toward fruits in the fall. Guglielmo is looking at how diet affects body composition and fuel use during flight.

For these experiments, he mixes up pans of casein- or sugar-rich agar and, after the mixtures harden, runs the gel through a food mill to make small, appetizing “worms.” Guglielmo calls the high-protein fare his Atkins diet for birds; those on it weigh about 2 grams less than birds feasting on high carbohydrates.

When Guglielmo first let the warblers try out the tunnel, they didn’t show much inclination to keep flying and instead kept landing on the upwind net. In earlier experiments, the researchers had tried everything—making that end of the tunnel dark, flashing strobe lights, even spraying water jets to discourage the birds from perching there. This night, they are pulling out all stops and taking the net down altogether. They tested a bird for 20 minutes and it didn’t head upwind too far, so now they are ready to see if the warblers will fly long distances. The next night, one bird lasted 45 minutes before landing on the tunnel floor—an okay flight time but not ideal. But the evening after that was a different story. “Purple-black,” named for the color tag on its leg, “found his groove and flew for 6 hours,” Guglielmo e-mailed the next day. “We had a great night.”

—ELIZABETH PENNISI

LETTERS

edited by Jennifer Sills

Recognizing Scientists
and Technologists

ON 17 NOVEMBER 2010, PRESIDENT OBAMA PRESENTED THE National Medals of Science and the National Medals of Technology and Innovation. These medals are the highest honor that the nation can bestow in science and technology, yet they are rarely mentioned by the popular media. Because Congress does not appropriate funds to implement the “outreach” of these medals, for many years the only national recognition was a private award ceremony with the President.

In 1991, George Rathmann, one of the founders of the biotech industry, facilitated the formation of what is now the National Science and Technology Medals Foundation. The mission of the Foundation is to promote the National Medal Laureates as role models for students and thereby encourage interest in science and math. To accomplish this goal, the Foundation hosts a banquet in conjunction with the White House ceremony. This banquet features videos highlighting the technical accomplishments of the Laureates, which then become the basis for stories that appear throughout the country.

Over the years, the Foundation has accumulated a wealth of electronic material on the Laureates, including biographies, interviews, and descriptions of their accomplishments (*1*). This recognition not only is a way to recognize the Laureates’ enormous efforts, but also serves to focus our attention on the seminal ideas in science, mathematics, and engineering. The stories behind these accomplishments often provide inspiration to others, which is essential to promote further achievements.

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1. National Science and Technology Medals Foundation (www.nationalmedals.org).



Health Organization in 2009 (*5*). Thus, although we have not achieved harmonized international standards, as has taken decades for other technologies, we are much closer than most people realize. We recognize the need to ensure that our enthusiasm for the promise of these approaches as powerful public health tools does not outstrip our responsibility to apply scientifically validated and socially acceptable product development practices. The tragedy would be if this important but complex birthing process were to stifle creativity in the development of not only genetics-based solutions, but all truly novel approaches that seek to reduce the serious health threat of diseases such as malaria and dengue fever. We hope that debates over specific circumstances do not cloud the urgent need for the development and deployment of new tools to mitigate these disease scourges.

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Genetics-Based Field
Studies Prioritize Safety

M. ENSERINK’S NEWS OF THE WEEK STORY ON the open release trials of genetically modified mosquitoes in the Cayman Islands (“GM mosquito trial alarms opponents, strains ties in Gates-funded project,” 19 November 2010, p. 1030) highlights the growing pains associated with bringing new technologies out of the laboratory into the field. Unlike for vaccines, drugs, and insecticides, no industry-wide standards are yet in place to guide either public or private efforts in the development

of these technologies. However, it is important for the public to know that the scientists working on these new technologies are aggressively supporting the formulation of best practices for their safe, efficient, ethical, and regulated application, and are reaching out to experts from a range of relevant disciplines for advice and counsel. A series of publications document the evolution of this process (*1–5*). Indeed, efforts are currently under way to develop a guidance framework for quality standards to assess safety and efficacy and to address regulatory, legal, social, and cultural issues, as recommended by an international consultation held at the World

Origins of Biodiversity

THE ORIGIN OF THE HIGH NEOTROPICAL biodiversity has been a controversial topic since Darwin. The debate has focused on the relative influences of the climate changes during the Pleistocene (the past

~2.6 million years) and the tectonic and geographical reorganizations that occurred before the Pleistocene (1). In their Review “Amazonia through time: Andean uplift, climate change, landscape evolution, and biodiversity” (12 November 2010, p. 927), C. Hoorn *et al.* conclude that the biodiversity patterns of the Amazon basin were largely shaped before the Pleistocene, facilitated by the Andean uplift. The authors dismiss Pleistocene diversification by arguing that, in the Neotropics, the refuge hypothesis (proposing that species diversified in isolated forests during glacial periods) has already been abandoned, and that fossils and molecular phylogenetics support mostly pre-Pleistocene diversification.

However, Pleistocene diversification could have resulted from a variety of mechanisms other than isolated forest remnants (2–4). Furthermore, Hoorn *et al.* cite my meta-analysis (5) in support of the pre-Pleistocene diversification, yet the conclusions I drew from that study contradict those of Hoorn *et al.*’s Review. I concluded that about half of the dated extant neotropical species originated during the Pleistocene and the other half before it, and that speciation proceeded in a continuous fashion with no evident bursts (5). In addition, phylogenetic evidence provided by Hoorn *et al.* is based on the dating of complexes of extant species (the crown clades) that in fact records the age of the oldest species within each group (6) but not necessarily the age of all the extant species, which should be necessarily younger. This overestimates pre-Pleistocene diversification. Earth’s biodiversity gradients are the result of a long and complex history of evolutionary trends, mediated by ecological processes and governed by external forces, in which not only speciation but also extinction should be considered, especially in extra-tropical areas (7).

The topic requires the synergy of many disciplines, in a wide range of spatial and temporal scales. Pleistocene speciation is one more element and should not be neglected; after all, we ourselves are a Pleistocene species barely 200,000 years old.

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Response

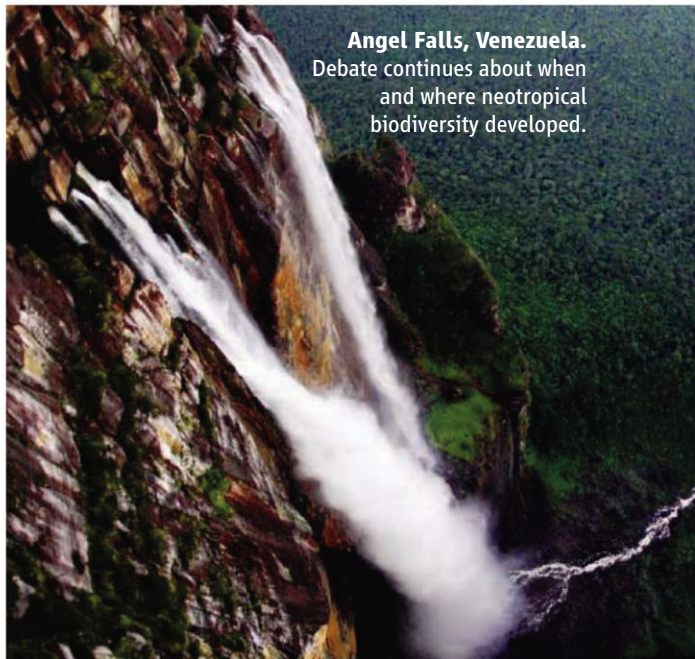
IN OUR REVIEW, WE LINK THE OUTSTANDING species richness in northern South America to the cataclysmic changes induced by Andean mountain building. Evidence for this is the correlation between sedimentary records, the paleontological record, dated molecular phylogenies, and present species distributions. Our conclusions contradict the hypothesis that has dominated for more than 40 years: that the outstanding levels of Neotropical species richness and current distribution patterns were mainly produced by Quaternary climatic fluctuations (1, 2), i.e., in the past 2.6 million years. All evidence in our meta-analysis points toward an older origin of Amazonian biodiversity.

Rull argues that we ignore Quaternary evidence on speciation, in part by erroneously referring to his previous meta-analysis (3) as evidence for pre-Quaternary diversification. Rull’s finding that about half of all extant species analyzed originated during Quaternary times (3) is not surprising. Assuming the average species longevity is some 100,000 to a couple of million years (3–5), at any point in time we would expect to find that most species originated in the past few million years. Rull’s evidence that extant species originated recently does not contradict the idea that the total

number of species was just as high (and for most organism groups higher) before the Quaternary, even if the species that existed then have since become extinct. Moreover, if Pleistocene glaciations had indeed produced most of the species richness observed today—as implied in the original formulation of the “refuge theory” (1)—this would unrealistically imply that all previous diversity was produced by entirely different mechanisms. This realization severely undermines the role of glaciation dynamics in accounting for Neotropical species richness.

Rull’s suggestion that we overestimated pre-Quaternary diversification by using genera instead of species as taxonomic units in our meta-analysis is misleading. Extinction is more likely to affect older lineages than younger ones—simply because species that have arisen recently have had less time to go extinct (6)—meaning that Pre-Quaternary speciation events were probably underestimated in Rull’s meta-analysis (3). Stochastic diversification models (6) can correct for the effect of background extinction in diversification rate estimates, but these models have proven unrealistic because of their oversimplified assumptions (7) and sensitivity to incomplete taxon sampling (8), a common feature in Neotropical phylogenies. Estimates of crown ages of genera are, arguably, less sensitive to incomplete taxon sampling, because in most species-level phylogenies, sampling is aimed to cover the geographic and morphological variation within a genus. This should lead to more robust age estimation of deeper nodes even when many species are missing.

Angel Falls, Venezuela.
Debate continues about when
and where neotropical
biodiversity developed.



The data we assembled show that the blueprint of present Amazonia was laid out in pre-Quaternary times, but they also have the potential to provide us clues on how the rainforest may react to future global warming. It is also clear that Amazonian biota withstood large geodynamic (9) and climatic fluctuations but that humans, the young product of Quaternary evolution, pose the biggest threat to this wealth of biodiversity.

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Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the past 3 months or matters of general interest. Letters are not acknowledged upon receipt. Whether published in full or in part, Letters are subject to editing for clarity and space. Letters submitted, published, or posted elsewhere, in print or online, will be disqualified. To submit a Letter, go to www.submit2science.org.

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CORRECTIONS AND CLARIFICATIONS

Perspectives: "The feeding habits of ammonites" by K. Tanabe (7 January, p. 37). The legend should read as follows with corrected genus names: "(Top) *Polyptychoceras* sp. with Baculites-like lower and upper jaws... (Bottom) *Anagaudryceras limatum* (a lytoceratid) with a nautilus-like lower jaw."

Policy Forum: "Boosting CITES" by J. Phelps et al. (24 December 2010, p. 1752). The heading for the fourth potential solution was missing. "A Peer-Review Process" should have appeared before the paragraph on the second page that begins, "CITES shortcomings may be overlooked because the convention lacks internal and external checks and balances." The header has been added in the HTML version online.

News Focus: "Will homebody researchers turn Japan into a scientific backwater?" by D. Normile (10 December 2010, p. 1475). There is a shift in the increments on the vertical axis of the graph indicating the number of individuals making overseas visits. From 0 up to 10,000 individuals, the graph uses increments of 2000; above 20,000, it uses increments of 20,000.

News of the Week: "U.N. biodiversity summit yields welcome and unexpected progress" by D. Normile (5 November 2010, p. 742). The name of Alison Stattersfield, head of science for BirdLife International, was misspelled.

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BEHAVIOR

Insect Swarm Intelligence

Lars Chittka and Alex Mesoudi

The swarming behavior of honeybees constitutes one of the most astounding phenomena in group decision-making among animals: When a new queen is raised in the honeybee hive, her predecessor departs along with approximately 10,000 workers. Rather than en masse erratically searching around for a new location (as a group of vertebrates might), the bee swarm does something remarkable. It acts, in a sense, like a single being: pausing, collecting information, carefully considering its options, and then making a unanimous decision about where to move. The consensus can take days to reach. Several hundred scouts fan out across a territory of up to 70 km² seeking a potential home, such as a hollow tree with a knothole entrance. Successful scouts return to the swarm and advertise the location of their discovery, but there is initially much disagreement among scouts and difference in the quality of sites found. The swarm must come to an agreement, however. Individual bees won't survive on their own, nor will groups of bees without their queen. If the polling about where to move takes too long, the swarm risks exposure to severe weather conditions that may spell the end of the whole endeavor. And if the group chooses a poor-quality site, the colony will not survive the winter. When Martin Lindauer, Nobel laureate Karl von Frisch's most successful student, first described the debate among scouts to his mentor, von Frisch exclaimed: "Congratulations! You have witnessed an ideal parliamentary debate; your bees can evidently change their decision when other scouts have to announce a better nesting site" (1). Having dedicated decades to the study of this unique, pluralistic decision-making process, Tom Seeley offers an engaging and fascinating account of it in *Honeybee Democracy*.

Seeley writes with infectious enthusiasm, and indeed there is much to be enthusiastic about. When he took over the study of collective bee decision-making from Lindauer in the 1970s, many mysteries remained. How is a cohesive relocation of several thou-

sand worker bees and a reluctant queen that hasn't seen daylight since her nuptial flight at least a year earlier initiated? How do scouts explore the landscape and evaluate the suitability of cavities for nesting sites? Back at the swarm, how do they convey the quality of their discoveries? How is agreement reached in the absence of any top-down, centralized moderation of the debate? What ensures that the consensus converges on the best option? What is the signal for lift-off? Once the swarm is airborne, how can some hundred informed scouts guide a "school-bus sized cloud," con-

taining several thousand individuals, over a distance of several kilometers to an inconspicuous knothole? Incorporating findings from innumerable ingenious experiments by the author, *Honeybee Democracy* includes answers to all the above questions.

Some ingredients of Seeley's approach are worth highlighting. He strongly advocates starting an investigation with the inductive (bottom-up) approach used by von Frisch and Lindauer before him. Carefully observing your study organism in its natural setting, taking everything in, you get to know your study organism thoroughly from many angles—and let unexpected or inexplicable phenomena pop out for you. Only then do you develop testable hypotheses and rigorous experiments to zero in on how particular processes might be explained. Surprisingly, even though many great behavioral biologists have adhered to this philosophy, it is now fashionable to use instead a top-down, hypothesis-driven approach: starting with what you and pretty much everyone else expects and either confirming or rejecting that. It is hard to see how one would ever explore genuinely new territory in this way. Seeley's simple message is, keep your eyes open for the unexpected.

Another ingredient of the author's success is the elegance and simplicity of his experiments. In times when researchers are often

assessed not by intellectual contribution or productivity but instead by the amount of funding they secure, Seeley shows that cutting-edge science can be produced with "equipment obtainable from the local shopping mall" (as Francis Ratnieks observes on the book's dust jacket). Indeed, many of the experimental procedures he used are simple like sushi (and equally exquisite). Some require considerable courage, however: one simultaneously exposed Seeley to the risk of falling from a tree, being attacked by angry bees, and being killed by cyanide gas. One senses that he carried out experiments in the golden age before the blight of "health and safety" and "risk assessment." The book is also pleasantly free of any pretense of an applied justification for the work. Although at one point Seeley inadvertently defused a tense cold war confrontation between the United States and the Soviet Union by pointing out that what was thought to be chemical weapons residue was actually bee excre-

Honeybee Democracy

by Thomas D. Seeley

Princeton University Press,
Princeton, NJ, 2010. 279 pp.
\$29.95. ISBN 9780691147215.



Pondering collective intelligence. Tom Seeley during a 1974 pilot study of a swarm choosing its home.

ment, his work was inspired entirely by the quest for scientific discovery. Funders and policy-makers today need reminding that pure, blue-skies research is a component of any healthy society.

Nonetheless, the work might have considerable implications beyond the question of how honeybee swarms move house. Seeley argues that human groups—juries, committees, governments—could learn from how bees make decisions. He suggests minimizing a leader's influence, allowing each group member to contribute their opinions in an independent and unbiased manner, and only reaching a single group decision once a

democratic quorum has been reached. Seeley even put these rules into practice as head of Cornell University's Department of Neurobiology and Behavior by minimizing his own influence, encouraging minority views, and making decisions through secret ballot.

Yet we should not ignore the disanalogies between human and bee decision-making. Human groups are frequently not united by common interest in the way that honeybee swarms are united by shared kinship. The former often comprise conflicting factions each fighting for their own self-interest. And when human groups do act as cohesive units, they are often too cohesive, with their members rarely acting as independent decision-

makers like honeybee scouts. Conformity prevents dissenting views and conflicting evidence from being considered, often with disastrous consequences—such as when NASA ignored warnings that a component on the Challenger shuttle was faulty and went ahead with its doomed launch (2). Moreover, whereas honeybee swarms are cooperative by virtue of shared kinship, groups of people are cooperative partly through conformity (3). Eliminating conformity may eliminate decision-making errors, but it may also reduce the cohesiveness that maintains human groups in the first place.

It is to Seeley's credit that he stimulates such a wide-ranging debate over the simi-

larities and differences in group decision-making among species. In addition, *Honeybee Democracy* offers wonderful testament to his career of careful investigation of a remarkable natural phenomenon. The breadth and depth of the studies reported in it should inspire all students of animal behavior.

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10.1126/science.1199780

BIOETHICS

An Embarrassment of Riches

Tim Lewens

Leon Kass—recently retired from the University of Chicago—chaired the President's Council on Bioethics (henceforth the Kass council) from its establishment by President George W. Bush in November 2001 until Kass stepped down in 2005. Kass's own academic work is sometimes regarded by scientists and ethicists as reactionary. He is known, for example, for his defense of what he calls “the wisdom of repugnance.” Conservative thinkers take this work to explain why we should heed the instinctive aversion that many people feel when confronted by new technological developments, whether those involve the overhaul of traditional parenting structures in the face of new reproductive technologies or the pharmaceutical enhancement of human bodies and minds. For others, there is no wisdom in repugnance itself: to claim otherwise is simply to offer a flimsy legitimization of irrational distaste in the face of progress.

The Kass council's reports, even more than Kass's own work, became, in Adam Briggles words, “a lightning rod for political controversy.” In particular, the council attracted criticism from many that its membership had been stacked to reflect Bush's

own conservative views and that it was insufficiently attentive to the existence of disagreement among its own members. In his brief and breezy *A Rich Bioethics*, Briggles (a philosopher at the University of North Texas) sets out to give an account of the council's fundamental conception of bioethics and to evaluate its performance against that conception.

Lest one think the book is an unadulterated apologia, let me say that Briggles's verdict on the Kass council is not uniformly positive. He argues, for example, that Kass's own appointment as chairman should have been conducted in a more open manner. He also argues that some of the council's reports were “noticeably deficient

when it comes to conveying dissent.” Briggles accepts that these factors justify some of the political criticism Kass's council received. However, his main goal is to vindicate the council's underlying vision of a “rich bioethics,” and he argues that in the main the council was true to that vision.

What, then, is rich bioethics? It has a number of strands, some very sensible, some far more questionable. It seeks to articulate the concerns that diverse constituencies feel when presented with technological innovations. It also involves a rather more sophisticated recognition that we cannot simply set society up in such a way that different individuals, with different ethical outlooks, can

all have equally good chances of living in the ways that they wish. The decisions that government makes, Briggles argues, cannot be thoroughly neutral across varying conceptions of the good life. This, again, is quite correct: Outlawing the use of cognitive-enhancing drugs affects the ability of those with a commitment to a certain vision of self-improvement to achieve their goals. If government permits such drugs to be used, broad societal changes (in terms of norms of performance, for example) negatively affect the lives of those whose commitment to a certain conception of “genuine” achievement makes them oppose the use of the drugs. For that reason, we need to be aware of the ways in which regulatory choices—even apparently “liberal” policies, which allow everyone a supposedly personal sphere of action—can promote some conceptions of a good life while disadvantaging others.

Briggles's rich bioethics becomes more questionable once we move beyond these foundational points. First, he seems to imply that while a bioethics council can articulate and explain the ethical standpoints of diverse groups, it is not appropriate for such a council to suggest pragmatic ways in which the ethical concerns of all might be reasonably, or partially, met. Yet even if one is convinced by Briggles's arguments that there is no wholly ethically neutral framework for the regulation of new technologies, it does not follow that a properly rich bioethics should refrain from defending pragmatic political solutions to the problem of reasonable conflict among those stances. Second, Briggles believes (if I understand him correctly) that the Kass council was the first to appreciate the importance of rich bioethics. But once we realize that bioethics can be at the same time decisive in its policy recommendations, mindful of the

A Rich Bioethics

Public Policy, Biotechnology, and the Kass Council

by Adam Briggles

University of Notre Dame Press, Notre Dame, IN, 2010. 230 pp. Paper, \$30. ISBN 9780268022211. Studies in Medical Ethics.

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importance of balancing diverse ethical concerns, and rich in its perception of the nature of those concerns, we will surely agree that many other national ethics councils (in the United States and elsewhere) have been just as rich in their treatment of pressing issues as Kass's was.

This brings me to a final point. Briggles' defense of the Kass council is most strained where he defends the propriety of appealing to species' natures as ethical yardsticks. He thinks that such natures exist and are reliably interpretable for all species: "The nature of *Danaus plexippus* [the monarch butterfly] is seamlessly descriptive and normative, as it defines what constitutes full flourishing within the pattern of that kind." If *D. plexippus* has a nature that defines what constitutes its flourishing, so *Homo sapiens* has a nature that defines what constitutes our flourishing. This neo-Aristotelian view can then be

wheeled in to justify a conservative opposition to new technologies that purport to violate or alter that nature.

Briggles moves far too quickly to justify this very contentious ethical framework. If human nature is really "seamlessly descriptive and normative," then one might think that by investigating human nature—i.e., by studying biology and psychology—the answers to fundamental ethical questions will be made plain. And yet it seems the very essence of ethical issues that people can understand the biological and psychological facts—relating to cognitive enhancement, say—while disagreeing about what should be done. Briggles would counter this by reminding us of his skepticism of the existence of any strict distinction between descriptive facts and ethical values. He is also skeptical of the view that ethical judgment is simply a matter of expressing one's preferences. Briggles

thinks this expressivist view cannot be reconciled with the fact that we can persuade others, on rational grounds, to change their ethical views. But for those who think that ethical standpoints can be sensitive to reasons, and even for those who deny the fact-value distinction, Briggles' notion of "right reasoning from nature" remains unclear, unless it simply means careful reasoning that takes into account the best available evidence.

Such reasoning is the sort of thing that everyone thinks they are doing, whether they are "transhumanists" (generally bullish about the ability of technology to improve our typical human capacities) or instead are the sorts of thoughtful conservatives who worry that valuable human institutions and practices will be defaced by such "progress." At the end of Briggles' book, one is left with a worry that the Kass council had no special claim to riches.

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DRUGS

"A Sin, a Crime, a Vice, or a Disease?"

People appear to have an almost-universal reliance on chemical agents to bring relief to the anxious state of being human. Such mind-altering drugs are the stuff of the latest exhibition at the Wellcome Collection in London, *High Society*, which continues through 27 February 2011. The exhibition offers a sober and neutral reflection on the often-controversial subject.

Curators Mike Jay, Caroline Fisher, and Emily Sargent guide visitors through an eclectic collation of artifacts, literary matter, and artworks—one typical of the wide-ranging approach we have learned to expect of this excellent museum.

The curators organized the exhibition around five topics that cover production, trade, self-experimentation, collective experiences, and social questions having to do with drug control. In addition to expected displays of tools required for using and abusing drugs and less-expected art installations, there are some extraordinary treasures. Lying among the large leather-bound books that the Wellcome seems to

own in profusion are two tiny clay tablets, dating from before the 6th century BCE. The Babylonian one describes the medicinal plant "qunnabu" (probably cannabis) used for gynecological conditions. In another case lies a sheet of bluish paper covered in thin writing and attendant ink blots: Samuel T. Coleridge's manuscript of the poetic fragment, *Kubla Khan*, which he claimed resulted

from a laudanum-induced dream. Many other fascinating things have been packed into the small exhibit space, making it worth devoting at least a couple of hours to examining them. Finally, to lull us into an interesting fog of introspection, the floor plan has visitors exit through a large cubicle projecting a panorama of opium poppies drifting in a breeze of soporific music.

Many of the items on display are also illustrated in the book that accompanies the exhibition. There, cultural historian Jay explores medical, religious, social, and economic roles psychoactive substances play around the world. His examples include coffee, kava, betel nuts, ecstasy, hashish, opium, and tobacco. He ranges from the

High Society

Mike Jay, Caroline Fisher, and Emily Sargent, curators

Wellcome Collection, London. Through 27 February 2011. www.wellcomecollection.org/whats-on/exhibitions/high-society.aspx

High Society

Mind-Altering Drugs in History and Culture/ The Central Role of Mind-Altering Drugs in History, Science, and Culture

by Mike Jay

Thames and Hudson, London, 2010. 192 pp. £18.95. ISBN 9780500251720. Inner Traditions, Rochester, VT. Paper, \$19.95. ISBN 9781594773938.



A popular pick-me-up. Dudley Hardy's ad (circa 1916) for "a marvelous restorative."

use of botanicals in antiquity through the self-experimentation of early scientists to possible designer neurochemicals. And he discusses the far-reaching consequences of international trade in intoxicants, prohibitions, and the "war on drugs." Whether or not they are able to visit the exhibition, readers will find the book provides an excellent overview of the cultural history of drugs. —Caroline Ash

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SCIENCE EDUCATION

Defeating Creationism in the Courtroom, But Not in the Classroom

Michael B. Berkman and Eric Plutzer*

Just over 5 years ago, the scientific community turned its attention to a courtroom in Harrisburg, Pennsylvania. Eleven parents sued their Dover, Pennsylvania, school board to overturn a policy explicitly legitimizing intelligent design creationism. The case, *Kitzmiller v. Dover*, followed a familiar script: Local citizens wanted their religious values validated by the science curriculum; prominent academics testified to the scientific consensus on evolution; and creationists lost decisively. Intelligent design was not science, held the court, but rather an effort to advance a religious view via public schools, a violation of the U.S. Constitution's Establishment Clause (1). Many scientists cheered the decision, agreeing with the court that the school board displayed "breathtaking inanity" [p. 765 (1)]. We suggest that the cheering was premature and the victory incomplete.

Systematic Undermining of Science

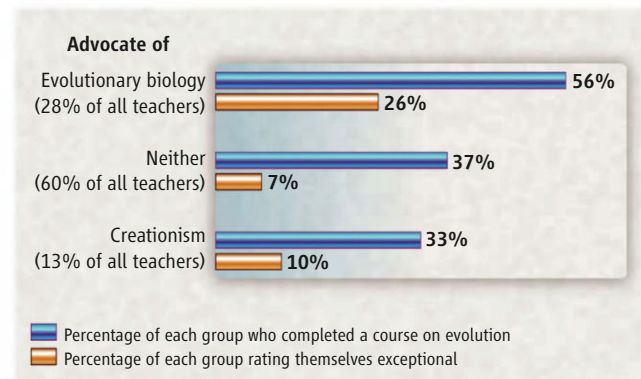
Creationism has lost every major U.S. federal court case for the past 40 years, and state curricular standards have improved (2). But considerable research suggests that supporters of evolution, scientific methods, and reason itself are losing battles in America's classrooms, where instruction in evolutionary biology "has been absent, cursory, or fraught with misinformation" [p. 21 (3), and (4)]. Extending this research, we have been investigating the evolution-creationism battle in state governments (5) and the nation's classrooms (2, 6). Central to this research is the National Survey of High School Biology teachers, based on a nationally representative probability sample of 926 public high school biology instructors (2, 6) (see the figure). [See supporting online material (SOM) for details.] The data reveal a pervasive reluctance of teachers to forthrightly explain evolutionary biology.

The data further expose a cycle of ignorance in which community antievolution attitudes are perpetuated by teaching that reinforces local community sentiment. For example, we ranked school districts from

least to most socially conservative, and in the 15% most socially conservative school districts, nearly 4 in 10 teachers personally do not accept human evolution (compared with 11% in the least conservative districts) and, consequently, devote only minimal time to evolutionary biology in their classes [table A8.2 in (2)]. The next generation of adults is thus predisposed to share the antievolution views of their parents.

More promising data suggest that America's high schools contain thousands of outstanding, effective educators of evolutionary biology. We estimate that 28% of all biology teachers consistently implement the major recommendations and conclusions of the National Research Council (7): They unabashedly introduce evidence that evolution has occurred and craft lesson plans so that evolution is a theme that unifies disparate topics in biology (2).

At the opposite extreme are 13% of the teachers surveyed who explicitly advocate creationism or intelligent design by spending at least 1 hour of class time presenting it in a positive light (an additional 5% of teachers report that they endorse creationism in passing or when answering student questions). The boldness and confidence of this minority should not be underestimated. Although 29% percent of all other teachers report having been "nervous at an open house event or meeting with parents," only 19% of advocates of creationism report this ($\chi^2 = 5.1$, $P = 0.024$).



Self-reports of qualifications of teachers, classified by approach to teaching evolution. Based on responses from 926 U.S. public high school biology teachers. See SOM for survey details.

Sixty percent of U.S. high school biology teachers are not advocates for either evolutionary biology or nonscientific alternatives.

Some advocates of creationism insisted that they—not bench scientists—are the ones practicing proper science: A Minnesota teacher commented, "I don't teach the theory of evolution in my life science classes, nor do I teach the Big Bang Theory in my [E]arth [S]cience classes.... We do not have time to do something that is at best poor science." Others rejected the possibility that scientific methods can shed light on the origin of species. An Illinois teacher responded, "I am always amazed at how evolution and creationism are treated as if they are right or wrong. They are both belief systems that can never be truly or fully proved or discredited."

The Cautious 60%

But if mainstream science and the modern creationist movement each have their classroom allies, they still account for only about 40% of all high school biology teachers. What of the majority of teachers, the "cautious 60%," who are neither strong advocates for evolutionary biology nor explicit endorsers of nonscientific alternatives?

Our data show that these teachers understandably want to avoid controversy. Often they have not taken a course in evolution and they lack confidence in their ability to defend it (see the figure, see SOM for details). Their strategies for avoiding controversy are varied, but three were especially common and each has the effect of undermining science (8). Some teach evolutionary biology as though it only applies to molecular biology—com-

pletely ignoring macroevolution of species. At best, this approach sacrifices a rich understanding of the diversity of species. At worst it lends credence to the creationist claim that there is no evidence for one species giving rise to others.

Others defend the teaching of evolution as a necessary evil, using state examination requirements as a convenient means to disassociate themselves from

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the very material they are expected to teach. These examinations have only been recently introduced in most states. Yet, many teachers told us that they tell students that it does not matter if they really “believe” in evolution, so long as they know it for the test. One Michigan teacher tells students that they need to understand evolution because the biology curriculum “is organized *as if* evolution is true” [emphasis added].

Finally, a sizable number of teachers expose their students to all positions—scientific or not. Students should make up their own minds, explained a Pennsylvania teacher, “based on their own beliefs and research. Not on what a textbook or on what a teacher says.” Many of these teachers might have great confidence in their students’ ability to learn by exploration. But does a 15-year-old student really have enough information to reject thousands of peer-reviewed scientific papers? This approach tells students that well-established concepts like common ancestry can be debated in the same way we debate personal opinions.

The cautious 60% may play a far more important role in hindering scientific literacy in the United States than the smaller number of explicit creationists. The strategies of emphasizing microevolution, justifying the curriculum on the basis of state-wide tests, or “teaching the controversy” all undermine the legitimacy of findings that are well established by the combination of peer review and replication. These teachers fail to explain the nature of scientific inquiry, undermine the authority of established experts, and legitimize creationist arguments, even if unintentionally.

Courts, Standards, Preservice Teachers

Biology will be the only high school science class for 21 to 25% of U.S. high school graduates, and more high school students take general biology than any other science course (9). But many are not afforded a sound science education, which is problematic in a democracy dependent on meaningful citizen input on highly technical, but consequential, public policies. Research suggests several ways that scientists and scientific organizations can address this situation.

First, continued participation in federal law suits is essential, as federal courts have been shown to limit effectively the ability of state and local governments to endorse non-scientific alternatives to evolution (5). Likewise, the active role of scientists and scientific organizations has improved curricular standards in many states, and such reform has the potential to be especially effective in states having high-stakes science tests

(2). But change due to improved standards is likely to be slow, because standards have the greatest impact on the newest teachers—those who were socialized in an era of standards-based education and who take standards and testing for granted (2). In addition, further improvements in state standards may be difficult because public opinion has been remarkably immune to outreach and public science efforts over the past three decades (10).

We suggest that increased focus be placed on preservice teachers (i.e., those preparing to be, but not yet, teachers). Teachers who are advocates for evolutionary biology are more likely to have completed a course in evolution than teachers who are ambivalent about evolution or who teach creationism (see the figure). Indeed, completing an evolution course is a powerful predictor of the classroom time devoted to evolution (6, 11) and the likelihood that teachers will integrate evolution into their class as a unifying theme (2). Many nonresearch institutions lack the resources to offer a stand-alone evolution course regularly, however, and such institutions educate many high school science teachers. Requiring an evolution course for all preservice biology teachers, as well as provision of resources to provide such a course, would likely lead to meaningful improvement in secondary school science instruction.

In addition to their relative lack of evolution coursework, teachers in the ambivalent middle 60% also resemble those who endorse creationism in that few believe that they have an exceptional understanding of evolutionary biology (see the figure). Yet, unlike creationists, few of these ambivalent teachers hold a young-Earth belief system (e.g., that the universe is only about 10,000 years old) that would prevent them from becoming strong advocates for evolutionary biology. Therefore, improving the instruction they receive in evolution as undergraduates is essential. Outreach efforts such as webinars, guest speakers, and refresher courses—the types of efforts currently aimed at secondary school teachers—could be tailored and targeted for both preservice teachers and for biology and science education professors at teaching-oriented colleges. This two-pronged effort may help increase the percentage of new teachers who accept and embrace the findings of evolutionary biology. Better understanding of the field should provide them with more confidence to teach evolution forthrightly, even in communities where public opinion is sympathetic to creationism.

More effectively integrating evolution into the education of preservice biology teachers

may also have the indirect effect of encouraging students who cannot accept evolution as a matter of faith to pursue other careers. Effective programs directed at preservice teachers can therefore both reduce the number of evolution deniers in the nation’s classrooms, increase the number who would gladly accept help in teaching evolution, and increase the number of cautious teachers who are nevertheless willing to embrace rigorous standards. This would reduce the supply of teachers who are especially attractive to the most conservative school districts, weakening the cycle of ignorance.

Outreach efforts primarily benefit teachers who want to be helped, so expanding the corps of science teachers who want to be helped is critical. Thus, focusing on the preservice stage may be “the most effective way for scientists to help to improve the understanding of evolution” [p. 332 (12)]. Better-trained teachers will be able to more effectively take advantage of details in their textbooks and supplementary material published by the National Academy of Sciences and to put aside fear of reactions and pressures from members of their communities. It would also make them more critical advocates for high-quality standards and textbooks. Combined with continued successes in courtrooms and the halls of state government, this approach offers our best chance of increasing the science literacy of future generations.

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COMPUTER SCIENCE

Cloud Computing—What's in It for Me as a Scientist?

Armando Fox

Many scientists would love access to large-scale computational resources but find that the programming demands of using a supercomputer—as well as the cost and queuing time—are too daunting. Privately owned cloud computers—large data centers filled with computers that mainly run their company's software—are now becoming available to outside users, including scientists and educators. Companies are leasing their computing resources on demand from a large shared pool to individuals who run their own software on a pay-as-you-go basis. This approach is an example of cost associativity (1): 1000 computers used for 1 hour costs the same as one computer used for 1000 hours. If your problem can be computed in a way that takes advantage of parallel processing, you can now get the answer 1000 times as fast for the same amount of money.

Although companies had long been operating “private clouds” that run programs such as Google Search or Microsoft Hotmail, Amazon was the first to let outside users run software on their computers. For example, Amazon's Elastic Compute Cloud (EC2), announced in late 2007, allows anyone with a credit card to use any number of computers in Amazon's data centers for 8.5 cents per computer-hour with no minimum or maximum purchase and no contract. Such an arrangement is possible because these “warehouse-scale” data centers (~50,000 servers, see the figure) are five to seven times cheaper to build and operate than smaller facilities (~1000 servers) (2), in terms of network, storage, and administrator costs. When operational costs, which include human administrators, power, and networking, are considered, the charges for cloud computing to outside users is price-competitive with using in-house facilities.

Cost-associativity enables new capabilities. For example, a researcher in our laboratory created an automated classifier to detect



spam on the popular social-communication site Twitter.com. Training the classifier took about 270 hours on a typical desktop workstation. The training program could be parallelized: The problem could be broken into pieces and run at the same time, only occasionally sharing results between different pieces. The same task took 3 hours using about 100 servers in Amazon's cloud (about \$250 in usage fees). Many universities have also begun to use cloud computing for education, where cost-associativity is a great fit for semester courses. Lots of computing demand could be provided around assignment deadlines (more than even the biggest schools could provide), and when demand is less (between deadlines), no outside resources need to be purchased.

Initially, cloud-computing hardware was configured primarily for its earliest adopters—Web-based applications—and early attempts to run scientific applications on the cloud gave discouraging results (3, 4). New hardware is now configured for better per-

Computational tasks that are inherently parallel, from simulations to student assignments, can be run faster on the data center resources of public clouds.

Commodity computing. This view of a Microsoft data center is a tiny fraction of the servers available for cloud computing.

formance on scientific applications. For example, Amazon's recently added “cluster computing instances,” priced at \$1.60 per computer-hour, run scientific benchmarks 8.5 times as fast as the original cloud hardware, according to experiments at the National Energy Research Scientific Computing Laboratory at Lawrence Berkeley National Laboratory (5).

Cloud computing works best when a problem can be broken down into a large number of relatively independent tasks, each running on its own computer. Software frameworks like Google's MapReduce (6) and its open-source equivalent Hadoop (7) provide a data-parallel “building block” for expressing such computations (much like a Web design framework allows you to “build” a Web site by filling in the relevant information and functions you want). Critically, these frameworks also hide the complex software machinery that handles inevitable transient machine failures when hundreds of machines in a cloud environment work on a problem simultaneously. Many of the “success stories” of science in the cloud have embraced Hadoop, and other popular tools such as the statistical package R now feature libraries that integrate with it. However, many problems cannot be easily expressed in terms of map and reduce tasks (mapping parcels out the work, and reduce collates the results). Even when they can, the programming effort required may be substantial.

Most desktop software is not written to take advantage of cloud computing and requires modification before it could harness cloud resources and run faster. However, the popular packages MATLAB and Mathematica are now available in versions that can “farm out” work to a public cloud. Cloud vendors including Amazon and IBM are working with independent software vendors on cloud-friendly versions of popular scientific software.

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What about software designed for parallel supercomputers? Programs written to the MPI (message-passing interface) standard run in the cloud, but the performance of such programs is sensitive to whether all the machines run in tight lockstep when working a problem (a characteristic of supercomputers but one that is not necessarily part of the cloud-computing architecture). Still, cloud technology suppliers such as Intel, Advanced Micro Devices, and VMware are adding hardware and software features necessary to improve MPI performance in the cloud, a move that highlights new “buying power” in the scientific computing community.

Many scientific computing problems do not require supercomputer performance but would benefit greatly from modest parallelism—say, tens or hundreds of computers in the cloud. The total “time to answer” may still be quicker, according to Foster (8), even for larger problems because a cloud-based supercomputer can be provisioned and running in minutes rather than hours or days, without waiting in a queue.

Some problems are so data intensive that they demand large-scale computing. The

Large Synoptic Survey Telescope in Chile may generate up to 30 terabytes of data daily. At typical long-haul network speeds of around 20 megabits per second (9), 30 terabytes would take more than 4 months and \$3000 in network charges to copy to Amazon’s cloud. Many cloud providers now allow users to ship crates of hard drives to be physically incorporated into the cloud infrastructure, an idea championed by the late Jim Gray.

The lure of improved performance has already drawn scientists and engineers to use cloud computing (10) in research on a number of topics, including large-population genetic risk analysis, information retrieval, and particle physics. Cloud computing installations are also being established by academic-industrial consortia [see, for example, (11–13)] to further encourage adoption of cloud computing by scientists.

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GENOMICS

The Genomic View of Bacterial Diversification

Mark C. Enright¹ and Brian G. Spratt²

Bacteria have unusual and variable sex lives, ranging from near-celibacy to evident promiscuity. “Sex” in bacteria involves a recipient bacterium replacing, by homologous recombination, small regions of its genome with corresponding regions from other strains (1). Studies over the past 20 years have demonstrated that in relatively promiscuous bacterial species, frequent recombination can drive rapid diversification of strains. In more celibate species, diversification is much slower and depends on the slow accumulation of point mutations (1, 2). Sorting out the roles that these processes play in the pace and pattern of bacterial evolution, however, has proven problematic. On page 430 of this issue, Croucher *et al.* (3) demonstrate a powerful new approach to the problem. By compar-

ing the genomes of many isolates of a single strain of a bacterium that causes pneumonia, they were able to rapidly obtain a comprehensive evaluation of the role that recombination, point mutation and other genetic processes played in its diversification. The approach offers insight into how this pathogenic strain can rapidly evolve resistance to antibiotics and evade future vaccines, and promises to help researchers better understand how this resistant strain may have spread globally.

It has been difficult to estimate the rates and patterns of recombinational replacements by comparing the genomes of distantly related strains of bacterial species, particularly when recombination has been frequent. Similarly, it has been problematic to identify those parts of the genome that have not been involved in recombination and can provide reliable information about phylogeny (the bacteria’s evolutionary history). Most of these problems can be over-

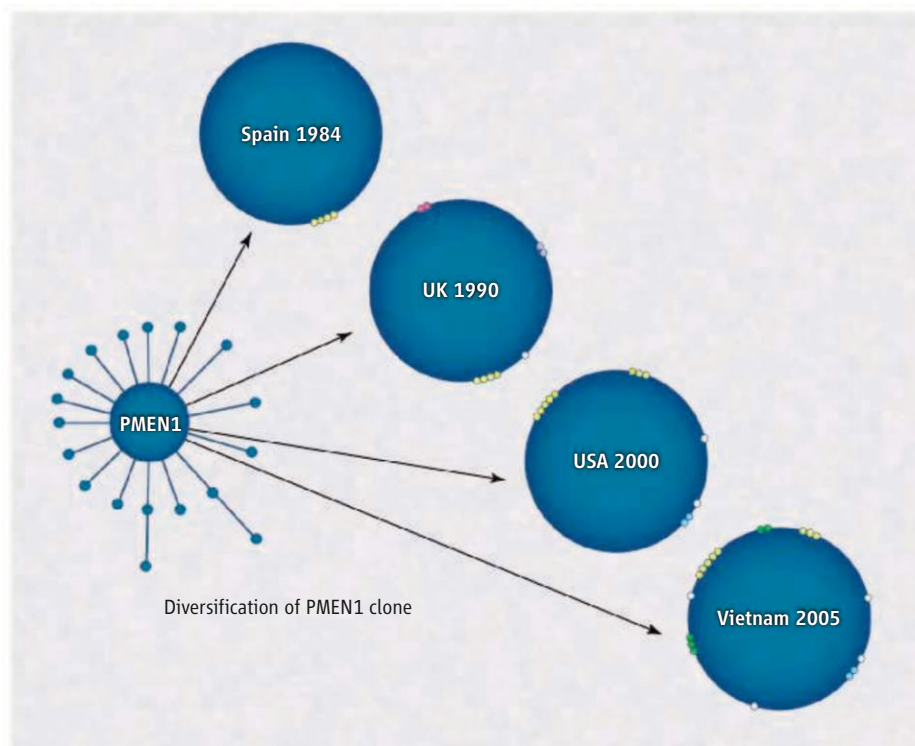
By comparing whole genome sequences, researchers reconstruct the evolution and global spread of an antibiotic-resistant strain.

come, however, by comparing the genomes of isolates that have a very recent common ancestor, such as isolates of an antibiotic-resistant strain of a bacterial pathogen, which must have emerged within the antibiotic era. These isolates will have accumulated a relatively small number of mutations and recombinational replacements, allowing researchers to more easily determine the number and size of the replacements. The ability to construct a reliable phylogeny, by identifying variation due to point mutations, is also greatly simplified. Differences among isolates can be mapped onto a tree and correlated with genomic differences, such as shifts in antibiotic resistance profiles or changes in virulence or antigens targeted by newly introduced vaccines.

Sequence data can provide information about the dynamics and geographic spread of pathogens. Such an analysis of very recent events, however, requires a pathogen that evolves rapidly enough to enable suffi-

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History of an antibiotic-resistant strain. Since its origin around 1970, the multiply antibiotic-resistant PMEN1 (pneumococcal molecular epidemiology network 1) clone of *S. pneumoniae* has diversified rapidly. PMEN1 is usually identified by multilocus sequence typing (MLST), which characterizes isolates using the sequences of seven genes. Most isolates of PMEN1 retain the ancestral sequences (central blue circle, left), but many isolates have arisen that differ in the sequence of one of the seven genes (around central blue circle, left) and two differ at two of the genes. Analyzing the genome sequences of 240 isolates of PMEN1 provides a comprehensive picture of this rapid genetic diversification of the antibiotic-resistant strain over 40 years. The genomes of four isolates of PMEN1 are represented (blue circles, right). Point mutations (single small white circles) and recombinational replacements (linked colored circles) are shown.

cient mutations to accumulate over the time scale of interest (4)—for example, during the rapid global spread of a new influenza pandemic (5). This requirement has largely limited the use of powerful new analytical methods to RNA viruses, which have very high mutation rates (4–7). Mutation rates in bacteria have been considered to be far too low to apply these new “phylogenetic” and “phylogeographic” approaches to reconstructing the geographic spread of a pathogenic strain over a matter of a few years or decades. Several research groups, however, have recently found that short-term mutation rates in various bacterial pathogens are much higher than previously believed (about 10^{-6} substitutions per site per year) (8–11). Although these rates are still much lower than those of RNA viruses, the much larger size of bacterial genomes, combined with the relatively high mutation rates, should allow researchers to apply the analytical methods pioneered for RNA viruses to bacterial pathogens, if whole genome sequences are used.

The ease with which investigators can

now obtain the whole genomes of bacterial pathogens is opening up a number of questions that previously were impossible or difficult to address. One of these is how virulent or highly drug-resistant strains of bacterial pathogens spread within hospitals or between hospitals and nursing homes within a region. Not surprisingly, the first application of the genome sequencing of large numbers of isolates of a single strain involved a study of the spread of a prevalent methicillin-resistant *Staphylococcus aureus* (MRSA) strain (11), and the preliminary results are encouraging.

Croucher *et al.* used large-scale genome sequencing to explore the evolutionary dynamics of a single multidrug-resistant strain of the community-acquired pathogen, *Streptococcus pneumoniae*. By sequencing 240 isolates, obtained from 22 countries between 1984 and 2008, they were able to track how the pathogen evolved over 40 years as it spread from continent to continent (see the figure). In this species, recombination is frequent: 74% of the genome

had received a recombinational replacement in at least one isolate. The genome-wide approach provides the first indication of the broad size distribution of these recombinational replacements, with 5% replacing more than 30 kb of the genome. Some of these replacements are responsible for changes in the bacteria’s capsular serotype and involve replacement of the large capsular polysaccharide biosynthetic locus. These changes almost certainly reflect selection by the human immune response or, recently, by the introduction of conjugate vaccines, which target a small subset of the 90+ known polysaccharide capsules. Several chromosomal regions were frequently replaced by recombination, including the *pspA* and *pspC* genes that encode surface antigens being developed as vaccine candidates.

By removing the recombinational replacements from the genomic data set, the researchers identified polymorphisms that they used to construct a phylogeny, allowing them to map the dynamics of the acquisition and loss of antibiotic resistance and of capsular serotype changes. Phylogeographic analysis supported a view that this multiresistant strain originated in Europe about 40 years ago, which is consistent with its initial recognition in Spain in the 1970s.

Overall, the large-scale genomic approach dramatically shows the remarkable ability of this pathogen to evolve over very short time scales by frequent recombination (and other processes). The consequences of such rapid evolution for the emergence and spread of antibiotic resistance and the evasion of vaccines targeted at single-protein antigens are clear.

Another striking indication of the ability of *S. pneumoniae* to evolve rapidly is provided by a recent study that sequenced the genomes of six isolates obtained over 7 months from the nasopharynx of a single child (12). During this period, the genome of a strain that colonized the child received an estimated 23 recombinational replacements, many of which were derived from a co-colonizing strain, resulting in the replacement of 7.8% of the genome (12).

Previous studies have already reached many of Croucher *et al.*’s conclusions (13, 14). Suggesting that we knew all this before, however, misses the importance of their study, in which a single experiment provided more information than has been achieved over 15 years of research. In contrast to previous work, which involved the analysis of a few genes per isolate, Croucher *et al.*’s genomic approach provides the complete story of how bacteria

diversify over the very short time scales that are of crucial importance for understanding and predicting the response of pathogens to new antibiotics and vaccines. It represents a new and powerful approach to obtaining a mass of information about the population and evolutionary biology of any bacterial (or archaeal) species at a modest and rapidly falling cost. Exciting times lie ahead for the integration of bacterial genome data, epidemiology, and evolution.

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MICROBIOLOGY

A Tail of Division

Alan F. Cowman and Christopher J. Tonkin

A common mechanism of pathogen survival is to invade and replicate within the protective niche of a host cell. This is true for apicomplexan parasites, a group of medically and agriculturally important pathogens that includes the malaria-causing *Plasmodium* spp., and *Toxoplasma gondii*, the causative agent of toxoplasmosis. An apicomplexan parasite penetrates the host cell plasma membrane and replicates only after invasion is complete. Although there is some understanding of how these pathogens recognize and invade host cells, little has been known about the molecular steps that initiate replication. On page 473 of this issue, Santos *et al.* (1) show that invasion and replication are inextricably linked through the function of a key apicomplexan parasite protein, Apical Membrane Antigen 1 (AMA1) (2–4).

Experiments with the more genetically tractable *T. gondii* have shown that AMA1 is released from the parasite's microneme organelles onto its surface. This is required for the subsequent release of lipid-rich rhoptry organelles into the host cell to form the parasitophorous vacuole that harbors and protects the pathogen from degradation (2). AMA1 functions in

invasion by binding to proteins (the RON complex) that the parasite inserts into the host cell plasma membrane (see the figure) (5). The AMA1-RON complex is associated with the “moving junction,” a contact point of the host cell and parasite plasma membranes through which the parasite gains traction to pull itself into the host cell.

AMA1 has a single transmembrane domain and a short, highly conserved cytoplasmic tail. It is thought that the cytoplasmic

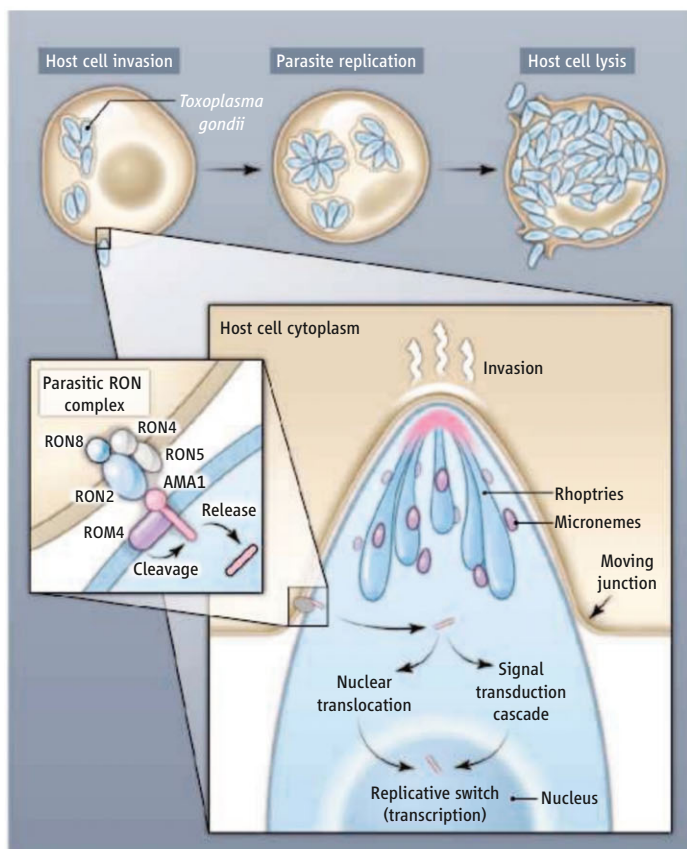
A protein that controls the entry of *Toxoplasma gondii* into a host cell also plays a role in controlling the parasite's replication.

tail could inform the parasite of contact with a host cell, thus triggering rhoptry release and activation of the parasite's actomyosin invasion motor (2, 4, 6, 7). Indeed, the cytoplasmic tail of AMA1 in *Plasmodium* plays a role in the invasion of erythrocytes (4), and its phosphorylation in *P. falciparum* is important in this function (6).

Cleavage of AMA1 is thought to be required for the parasite to release the host cell plasma membrane and complete invasion. In both *T. gondii* and *Plasmodium*, serine proteases called rhomboids (8) cleave AMA1 (and other parasite proteins that function in adhesion to the host cell) distal to the membrane, a prerequisite for successful invasion (9, 10). Indeed, the absence of Rhomboid 4 (ROM4) in *T. gondii* prevents shedding of the parasite's surface proteins, and a decrease in ROM4 function interferes with the apical-poste-

Linking invasion and replication.

To invade a host cell, AMA1 protein is secreted by the parasite *T. gondii* and inserted into the pathogen's cell surface membrane. It binds to the parasite-derived RON complex (RON2, RON5, RON4, and RON8) that has been inserted into the host cell plasma membrane. The protease ROM4 is located in the parasite's plasma membrane, where it cleaves AMA1 in the transmembrane region, thus releasing the AMA1 tail into the cytoplasm. The AMA1 cytoplasmic tail may interact with factors that trigger a signaling cascade, and/or itself translocate into the nucleus, to initiate the replicative transcriptional program.



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rior distribution of cell surface proteins that interact with host cell surface proteins, thus impairing the invasion process (9).

Santos *et al.* reveal another critical function of AMA1. They show that cleavage of AMA1 by ROM4 in *T. gondii* releases the cytoplasmic tail of AMA1, which then provides a signal for the invading parasite to begin replication inside the host cell. The finding indicates that invasion and replication in apicomplexan parasites are intimately linked. The authors demonstrate this important finding by constructing a *T. gondii* parasite that expressed a catalytically inactive form of ROM4 (ddROM4_{S-A}) (11). The mutant protein acted as a dominant negative and inhibited host cell invasion, confirming other work (9). However, the main effect of ddROM4_{S-A} expression was to block parasite replication. These parasites were blocked late in their cell division cycle (during S phase).

Santos *et al.* hypothesized that the AMA1 cytoplasmic tail released by ROM4 initiated asexual replication. Expression of the cytoplasmic tail rescued the cell division phe-

notype in parasites expressing the inactive form of ROM4, thereby allowing parasites to develop normally. Expression of the AMA1 cytoplasmic tail of *P. falciparum* also rescued the aberrant cell division phenotype, showing that this effect of AMA1 is a general function in apicomplexan parasites. A serine residue in the AMA1 cytoplasmic tail of *P. falciparum* is required for invasion and is phosphorylated (4, 6). Santos *et al.* found that mutation of this serine did not affect parasite cell division, indicating that the cytoplasmic tail of AMA1 has dual functions in invasion and initiation of the replicative phase of its life cycle.

AMA1 and ROM4 are both on the surface of extracellular parasites and at this stage, replication is suppressed. This suggests that a level of control must exist to govern the switch to replication only upon invasion. Could the activity of ROM4 be regulated to prevent AMA1 cleavage? This seems unlikely, as ROM4 activity in extracellular parasites has been inferred previously (9). Furthermore, Santos *et al.* also found that the AMA1 cytoplasmic tail alone cannot induce replication of

extracellular parasites, suggesting that other regulatory mechanisms must exist.

How does AMA1 promote replication? Possibilities include initiation of a signaling cascade, or directly influencing transcription machinery upon translocation into the nucleus. The identity of factors that associate with the AMA1 tail to drive two processes—replication and invasion—will be of great interest in the future.

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CELL BIOLOGY

Why Starving Cells Eat Themselves

D. Grahame Hardie

In the 1950s, electron microscopists observed regions of cytoplasm and organelles being engulfed by a double membrane to form vesicles that then fused with lysosomes (1). This process, called autophagy (“self-eating”), was correctly deduced to be a mechanism to degrade and recycle the contents of nutrient-starved cells (2). Since then, signaling pathways that sense nutrient deprivation and energy stress have been identified, but how these interact with autophagy has not been clear. On page 456 in this issue, Egan *et al.* (3) show that the adenosine monophosphate-activated protein kinase (AMPK), an energy sensor that is also activated by glucose starvation, triggers autophagy by phosphorylating ULK1, establishing a direct molecular link between the two processes.

The autophagy gene *ATG1* (originally *APG1*) was identified in the yeast *Saccharomyces cerevisiae* through mutations that led to failure of autophagic vesicles to accumulate when cells were starved (4). The two orthologs of Atg1 in mammals, ULK1 and ULK2, are the catalytic subunits of multisub-

unit protein kinases (also containing ATG13 and FIP200) that initiate the autophagic cascade. A key cellular nutrient sensor is the target of rapamycin complex-1 (TORC1), which is activated by amino acids via the Rag guanosine triphosphatases (GTPases) (5). Conversely, amino acid starvation inhibits TORC1 and promotes autophagy, as does the TORC1 inhibitor rapamycin. TORC1 is inhibited by AMPK (6, 7), and it has been generally assumed that this is how energy stress or glucose starvation promotes autophagy. However, Egan *et al.* now show that AMPK also achieves this by directly phosphorylating ULK1.

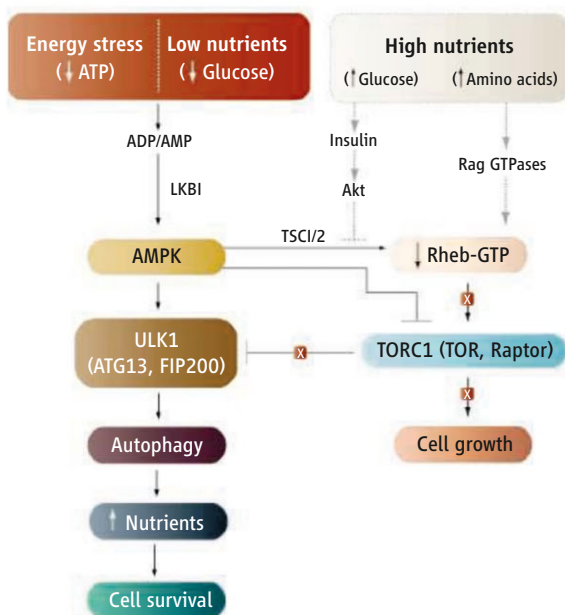
In mammals, increases in the ADP:ATP and/or AMP:ATP ratios, which indicate an energy deficit, are the signals that activate AMPK (8). The yeast ortholog is activated by glucose starvation, although whether changes in adenine nucleotides are the key activating signals (9) remains unclear.

To identify new targets for AMPK, Egan *et al.* looked for the conserved sequence motif that AMPK recognizes in its substrates (7, 10). Phosphorylation by AMPK often creates binding sites for 14-3-3 proteins, so the authors also searched for proteins that bound

In cells starved of nutrients, the process of autophagy is induced to recycle surplus cellular components to support cell survival.

to 14-3-3 proteins when AMPK was activated. This two-part screen identified four potential sites in the central domain of ULK1 (Ser⁴⁶⁷, Ser⁵⁵⁵, Thr⁵⁷⁴, and Ser⁶³⁷), of which two (Ser⁵⁵⁵ and Thr⁵⁷⁴) are conserved in ULK2. Supporting this connection, a screen for proteins that interact with ULK1 or ULK2 had identified six of the seven subunit isoforms of AMPK (11), suggesting that AMPK and ULK1 or ULK2 form stable complexes.

Using mass spectrometry and antibodies against specific phosphorylation sites, Egan *et al.* show that three of the ULK1 sites were phosphorylated by AMPK in cell-free assays, and that Ser⁵⁵⁵ was phosphorylated in wild-type, but not AMPK-deficient, cells treated with an AMPK activator. To determine whether phosphorylation at one or more of the sites is required to induce autophagy, the authors generated a “4A” mutant in which all four serine and threonine residues were changed to alanine. Wild-type ULK1 or the 4A mutant was expressed in cultured human cells in which the amount of endogenous ULK1 had been reduced by RNA interference, or in ULK1-deficient mouse embryo fibroblasts. These approaches revealed that various markers of autophagy were reduced



in cells expressing the 4A mutant, either under normal culture conditions or during nutrient starvation. Egan *et al.* claim that the Ser⁵⁵⁵ and Thr⁵⁷⁴ sites are conserved in the nematode *Caenorhabditis elegans* ortholog Unc-51, although this can be debated (12). They do provide evidence that the *C. elegans* ortholog of AMPK (Aak-2) triggers autophagy, although the role of phosphorylation of Unc-51 was not addressed.

Although further work is required to delineate the roles of the individual phosphorylation sites, the findings of Egan *et al.* suggest that the AMPK signaling pathway uses a “belt and braces” approach to activate autophagy in mammals, using both phosphorylation of ULK1 and inhibition of

the AMPK sites are conserved. Indeed, there is little current evidence that the yeast AMPK ortholog (the SNF1 complex) acts upstream of TOR, although there is evidence that it regulates autophagy. In *snf1* mutants the increase in autophagy induced when cells enter stationary phase due to glucose depletion is defective, whereas both *ATG1* and *ATG13* suppress the phenotype of low glycogen accumulation observed during stationary phase (13). Thus, the “belt” of activation of ULK1 (Atg1) by AMPK (SNF1) may have evolved early, whereas the “braces” of inhibition of TORC1 by AMPK may have arisen later. One can only speculate as to why this might have happened, although the role of the AMPK complex itself also

Pathways linking nutrients and autophagy. Under conditions of energy stress or glucose starvation, mammalian cells activate AMPK, which then phosphorylates and activates the ULK1 complex. AMPK also inactivates TORC1 by phosphorylating Raptor as well as by phosphorylating and activating TSC2 (converting Rheb to its inactive, GDP form). Signaling pathways that are blocked by AMPK activation under these conditions are marked with a cross. Glucose availability, acting in metazoans via insulin and Akt, activates TORC1 by phosphorylation and inactivation of TSC2. Amino acid availability activates TORC1 via the Rag GTPases. Thus, the ULK1 complex triggers autophagy during nutrient starvation and energy stress, whereas TORC1 inhibits autophagy and stimulates cell growth in the presence of nutrients and sufficient energy.

TORC1 (see the figure). AMPK inhibits TORC1 by phosphorylating its upstream regulator TSC2 (6) and the TORC1 subunit, Raptor (9). However, some of these mechanisms are not conserved in lower eukaryotes. Thus, there is no ortholog of TSC2 in *S. cerevisiae*, and although there is a Raptor ortholog (Kog1), because of a low degree of sequence conservation it is not clear whether

appears to have changed. In yeast, the SNF1 complex is probably mainly concerned with the response to glucose starvation, but for mammalian cells the ability of AMPK to sense energy status may have become more important. Perhaps inhibition of TORC1 by AMPK evolved as a mechanism to ensure that autophagy remains active and growth repressed during energy stress, even though amino acids are available.

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CHEMISTRY

Chemical Kinetics Under Test

Millard H. Alexander

The dependence of reaction rates on the isotopic identity of the reactants and products, called the “kinetic isotope effect” (1–3), is a manifestation of the role quantum zero-point energy plays in chemical kinetics and is a consequence of the Born-Oppenheimer (BO) separation of electronic and nuclear motion in molecules (4, 5). On page 448 of this issue, Fleming

et al. (6) use muon chemistry to probe the range of nuclear masses over which this approximation is valid for the chemical reaction $\text{H} + \text{H}_2 \rightarrow \text{H}_2 + \text{H}$ (7).

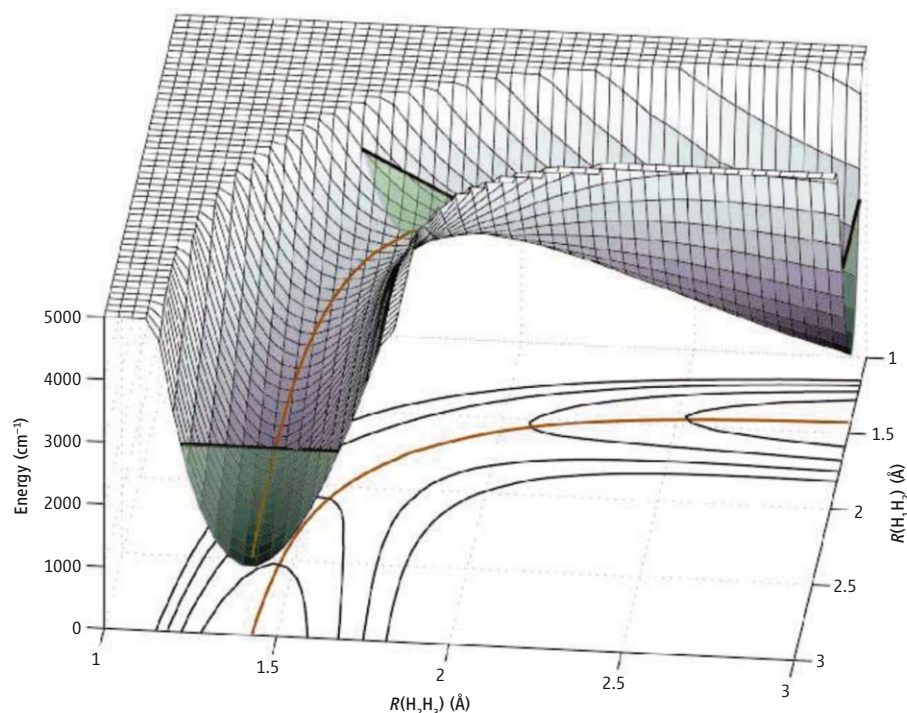
The BO approximation makes possible the practical application of quantum mechanics to all of molecular science. As the arrangement of the nuclei changes, the BO approximation postulates that the electrons will remain in a particular quantum state. The practical implication is that any molecule, consisting of a number of nuclei and, usually, a larger number of electrons, can be treated

A wide range of hydrogen and helium isotopes serve as a fundamental test of isotope effects and tunneling in chemical reactions.

quantum mechanically in two stages. First, for a given nuclear arrangement, one determines the energy of the electrons, usually in their ground electronic state. The gradient of this electronic energy (along with the electrostatic repulsion between the nuclei) then determines the forces on the nuclei. This two-stage separation enables the understanding of molecular spectroscopy as well as the simulation of the molecular dynamics in systems ranging from the simple to the complex.

One direct consequence of the BO approximation is the understanding of an

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Mapping out a reaction. Surface mesh plot of the potential energy surface for the collinear $H_1 + H_2H_3 \rightarrow H_1H_2 + H_3$ reaction as a function of the two bond distances, R , superimposed on a conventional contour plot of the potential energy surface. The minimum-energy reaction path is indicated by the solid orange line. The zero-point energy in the reactant and product arrangements, and at the barrier, is indicated by straight black lines. The three corresponding green slices indicate the range of coordinate space made classically accessible by zero-point energy.

important quantum effect in chemical kinetics: the kinetic isotope effect (1–3). Consider the simplest diatomic molecule H_2 and its isotopomer HD (hydrogen deuteride). Within the BO approximation, both H_2 and HD have the same electronic Hamiltonian and, thus, the same potential energy curves. However, because their nuclear reduced masses are so different [0.50 atomic mass units (amu) for H_2 and 0.66 amu for HD], the vibrational spacing differs dramatically. In particular, the quantum zero-point energy of H_2 is 33% larger than that of HD.

Especially in the case of H transfer and exchange, the dependence of zero-point energy on the isotopic identity of the reactants and/or products in a chemical reaction can lead to large differences in equilibrium constants and chemical reaction rate constants (1–3). The isotopic variation of the zero-point energy will affect not only the reaction exothermicity but also the height of the transition state—the activation energy. The magnitude of the rate constant is proportional to the density of vibration-rotation states of the reaction complex at the transition state, which also depends on the isotopic masses (1).

A subtle consequence of differing isotopic masses on chemical reaction rates can be

seen in terms of the potential energy surface for the $H + H_2$ reaction (see the figure). The appreciable zero-point motion of the light H atom implies that the reaction occurs not just along the reaction path, which is the minimum-energy pathway depicted in the figure, but also over a sizable region of configuration space around this minimum path, as indicated by the green slices. Isotopic substitution will control the width of the slice of the potential energy surfaces sampled during the reaction.

Fleming *et al.* report the study of $H + H_2$ reactivity over the widest possible range of isotopic masses. First, they have succeeded in replacing the H atom with muonium (an electron orbiting a positive muon, μ^+). Within the BO approximation, this behaves as a very light H atom with a mass of only 0.11 amu. On the other end of the scale, they have replaced one of the electrons in the ^4He atom with a negative muon, μ^- . Because μ^- is 207 times heavier than the electron, it orbits the He nucleus at a very close range, effectively screening one of the protons. Thus, the $^4\text{He}\mu$ system behaves chemically as a very heavy H atom with mass of 4.1 amu.

The rate constants from these unique experiments, combining accelerator physics

and chemical kinetics, are compared with full quantum-mechanical reactive-scattering calculations (8), based on a very sophisticated BO H_3 potential energy surface (9), and with the result of a variational extension of transition state theory (10), in its latest form (11). Excellent agreement is found with experiment over the entire range, in which the mass of the H atom is varied from 0.11 amu, through 1 amu (the mass of the normal H atom), and then to 4.1 amu. The tests reported by Fleming *et al.* over a large range of isotopic masses thus verifies the BO separation of electronic and nuclear motion in the paradigm $H + H_2$ reaction. Further, the comparison between the exact quantum treatment of the nuclear dynamics and (modified) transition state theory has shown that the latter accurately describes the subtle variation of chemical reactivity with the isotopic mass of the reactant, at least in the $H + H_2$ reaction.

The quantitative agreement between theory and experiment seen by Fleming *et al.* confirms our ability to predict with high accuracy the fine details of potential energy surfaces for small triatomic reactions and to model the details of the reaction dynamics.

However, a breakdown in any approximation is always possible. Recent work (12, 13) has delimited the small, but measurable, BO breakdown in the simplest chemical reaction beyond $H + H_2$, namely $F + H_2 \rightarrow FH + H$ and its analog $Cl + H_2 \rightarrow ClH + H$. One wonders whether it would be possible to find a triatomic reaction with a barrier in which the dependence of the rate constants (or, in more detail, the cross sections) on the isotopic mass of the reactants deviates from the predictions of transition state theory.

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SPORE* SERIES WINNER

Penguins and Polar Bears Integrates Science and Literacy

Jessica Fries-Gaither† and Kimberly Lightle

Science is often not included in elementary school curriculum despite the recognized importance of early development of science concepts and skills (1–3). In addition, there are few science resources available online that are focused on elementary school students. This combined state of affairs inspired the creation of an online magazine for elementary school teachers and their students: *Beyond Penguins and Polar Bears*.

Launched in March of 2008, *Beyond Penguins and Polar Bears* (<http://beyondpenguins.nsdl.org>) consists of 20 thematic issues relating elementary science concepts to the real-world context of the polar regions (see the first figure). *Beyond Penguins and Polar Bears* presents science content for topics such as rocks and minerals, the water cycle, seasons, states and changes of matter, plants, the indigenous peoples of the Arctic, polar research and explorers, and climate change in the context of life in the Arctic and Antarctica.

The online magazine format provides professional development content and instructional resources with a focus on integrating science and literacy. The strategy was initially conceived as a “Trojan horse” approach, using the reading and writing that elementary teachers were comfortable with as a vehicle for increased science instruction.

As the project progressed, it became apparent that the overlap between science and literacy was much richer than originally anticipated. Inquiry-based instruction and hands-on experiences help students develop the background knowledge needed to comprehend science texts. These experiences also build vocabulary and provide an authentic purpose for reading and writing (4). Reading about science concepts complements and extends the knowledge that students gain from inquiry and hands-on experiences and can substitute for direct experience when that is not possible (5). Writing and discussion around scientific concepts



Beacon Valley field camp, Antarctica.

allow students to share their knowledge as members of a scientific community in the same way practicing scientists do (6). Evidence suggests that such a combined curricular approach can benefit the development of students’ reading engagement and comprehension, academic language, and written and oral discourse abilities (7–9). There is also growing recognition that students’ ability to engage in the authentic practice and process of science requires the development and use of the structures of logical argumentation and the ability to read and write in informational text genres (10, 11).

The content of each issue of *Beyond Penguins and Polar Bears* is organized into five departments:

- *Professional Learning* contains resources for teachers, including science and literacy content knowledge, student misconceptions in science, teaching and assessment strategies, and information about creating an equitable classroom environment.
- *Science and Literacy* includes lesson plans and a virtual bookshelf of children’s literature.
- *Across the Curriculum* includes ideas for integrating other disciplines—such as

Elementary teachers find resources about the Arctic and Antarctica along with practical ideas for enhancing children’s literacy skills in this online project.

math, social studies, and art—into a science lesson or unit.

- *In the Field: Scientists at Work* profiles polar researchers.
- *Polar News and Notes* provides updates on news, research, and opportunities for teachers and students.

Beyond Penguins and Polar Bears also includes original content. A monthly nonfiction article, called a feature story, is written for students and is available in three grade bands, kindergarten to grade 1 (K to 1) and grades 2 to 3 and 4 to 5. Teachers can print full-color books with illustrations, access electronic versions with recorded narration, or print a text-only version of each story. Unit plans help teachers assemble the resources available in a given issue into a cohesive instructional framework based on the 5E learning cycle (engage, explore, explain, elaborate, and evaluate), a model used to plan inquiry lessons. All resources are correlated to the National Science Education Standards and/or the National Council of Teachers of English Language Arts and International Reading Association’s Standards for the English Language Arts.

Pilot testing of project materials with 19 teachers and 173 K to 5 students in

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*SPORE, Science Prize for Online Resources in Education; www.sciencemag.org/special/spore/.

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Columbus, Ohio, and Charlotte, North Carolina, was conducted over a 2-year period. Teachers and students were different in every year of testing. Teachers were asked to include resources from Beyond Penguins and Polar Bears as either a supplement to or a replacement for the existing curriculum, to keep a journal of the Web site and resource usage, and to participate in classroom observations, interviews, and pre- and post-teaching questionnaires.

Data that were collected assessed teacher usage of reform-based teaching practices, including the integration of science and literacy instruction, utilization of Beyond Penguins and Polar Bears, and changes in classroom practices following integration of the resources therein. Teachers reported changes, such as an increase in their likelihood to provide opportunities to read about science and to have their students write to communicate scientific results. Teachers also reported that an increased content knowledge about the polar regions and science in general also increased their confidence in teaching science (12).

Student questionnaires assessed attitudes regarding science. Statistically significant changes were seen in third grade students who were less likely to agree with the statements that “science was mostly memorizing facts” and that “science is more for boys than girls.” They were also more likely to agree that writing is important in science (13).

In subsequent pilot testing, another group



Northern lights, Greenland.

of third grade students was significantly more likely to agree with the statements “I like science,” “I am good at science,” and “I understand more of what goes on in science” (12). Over the entire pilot testing period, students were more likely to agree with the statements “I am good at science” and “writing is important in science.” Students in fourth and fifth grade did not show significant changes in their agreement with these statements after exposure to project materials. Continued evaluation efforts include a closer look at why third grade might be a critical point in developing students’ understanding and attitudes about science.

Beyond Penguins and Polar Bears capitalizes on social media, such as Facebook (Beyond Penguins) and Twitter (@beyondpenguins), to connect teachers to project content. Monthly Web seminars provide opportunities for virtual professional development in science and literacy instruction and exposure to project resources. Archives and descriptions of upcoming seminars are available on the home page and at <http://bit.ly/BPPBseminars>. Such seminars have received positive evaluations from participants, who have included classroom teachers, curriculum specialists, and other education professionals.

Although our goal was to provide an online resource that could be easily accessed across the country and around the world, we also realized that many teachers still prefer printed versions of resources. We therefore provide a “print-on-demand” option (see <http://bit.ly/printondemand>).

The Arctic and Antarctica are remote, beautiful, unique, and fragile (see the second figure). Through Beyond Penguins and Polar Bears, we hope that we have brought these far-off places a little closer to elementary teachers and students and have inspired excellent science instruction, too.

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team from The Ohio State University College of Education and Human Ecology; the Ohio Resource Center for Mathematics, Science, and Reading; the Byrd Polar Research Center; the Columbus Center for Science and Industry (COSI); the Upper Arlington Public Library; and the National Science Digital Library (NSDL). Experts in science and education fields, including climatologist Andy Monaghan of the National Center for Atmospheric Research (NCAR), paleomammologist Ross MacPhee of the American Museum of Natural History (AMNH), and Mark McCaffrey of the Cooperative Institute for Research in Environmental Sciences (CIRES), served as guest columnists.



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PRESIDENTIAL ADDRESS

Life on the River of Science

Peter Agre

THE YEAR 2010 MARKED THE CENTENNIAL of Mark Twain's death. More than any other American author, Twain exemplified the use of personal anecdotes to illustrate events in our nation's history. With this in mind, I will attempt to share my experiences in science, beginning one half-century ago, with a view for how we as individuals are part of the great river that science has become.

It is my hope to stimulate young scientists and inform the nonscientific public that achieving success in science involves several features. But if my experience is representative, the most important features include basic curiosity, the will to take chances, and the generous attention of family members and teachers. Probably for most individuals who became scientists during this time, guarantees of financial success were not considered, and with no large fortune to lose, it is no surprise that many scientists came from America's large middle class.

Science and Growing Up in the 1950s

While I was a child in the 1950s, "science" was a word familiar to American schoolchildren. Just a decade earlier, the end of World War II, brought about by dropping atomic bombs on Hiroshima and Nagasaki, Japan, made everyone aware that we had entered the nuclear age. Because the polio epidemic had touched tens of thousands of American families, the name of vaccine pioneer Jonas Salk was universally recognized.

The widespread introduction of television into American homes in the 1950s allowed American children to see the special magic of science by watching Don Herbert as "Mr. Wizard" on Saturday mornings. On Sunday evenings, children viewed the Disney show, whose format reflected the four kingdoms of the Disneyland amusement park, one of which, Tomorrowland, focused on science.

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Although it occurred more than 50 years ago, I distinctly recall Wernher von Braun discussing rocketry and space travel. In an unforgettable Disney program, University of California Berkeley Nobel laureate Glenn Seaborg provided a remarkable demonstration of the chemical chain reaction. Holding a spring-loaded mousetrap with a ping-pong ball on it, he sprung the trap and the ball flew. The camera then panned a room with the floor covered with activated mousetraps, each with a ping-pong ball on it. Seaborg tossed in one ping-pong ball, and within seconds the room was a cloud of flying mouse-traps and ping-pong balls.



Seal River enters Hudson Bay, 2004

Of course, actual hands-on science trumped even television, and having a father who was head of the chemistry department at St. Olaf College, a small liberal arts school in the rolling farmlands of southern Minnesota, gave me opportunities available to few children. My brothers and I marveled as we added a drop of a colorless solution to a beaker containing another colorless solution that instantly turned brilliant pink. Addition of a drop from a third solution caused the pink color to disappear again. What we first viewed as "magic" became understandable when we learned about alkali, acid, and indicator dyes. Marked by the experience, I recall our third-grade teacher asking us to draw ourselves as adults performing our life's work. I proudly drew a picture of a

chemist holding test tubes, since I wanted to be like my father—a chemist.

On a Saturday afternoon in October 1957, Dad came across the meadow to where my brothers and I were playing ball to bring us home for dinner. As we walked, he spoke of the breaking news story on the radio: the launching of Sputnik. We scanned the sky and failed to see the satellite, but it was no less real. More than any other single event, the launching of Sputnik began a remarkable renewal of the already strong American support for science. The motivation was based on the national humiliation of being beaten into space by our adversary, the Soviet Union, but the outcome was very positive.

The outpouring of funds for science and science education affected us directly. Dad wrote a National Science Foundation Fellowship that allowed us to move to Berkeley, California, for a sabbatical year at the University of California. Perhaps resembling the Nor-

wegian equivalent of the Beverly Hillbillies, we packed our old Chevrolet station wagon for the drive across the country. We arrived in Berkeley, a forward-thinking, multicultural community markedly unlike our quiet farming community in southern Minnesota.

A Family's Scientific Hero

Hero figures are important in the development of a child. During our year in California, we became familiar with Dad's new colleagues, including a chemist from Caltech with whom Dad served on the American Chemical Society Education Committee.

Linus Pauling had an exuberant personality, and we got to know him when he stayed at our house. Eating cornflakes at the breakfast table with the tall, grinning Pauling, wearing

CREDIT: BOB FRENCH

a black beret over his curly white hair, was simply unforgettable. Dad always beamed about Pauling, who had received the 1954 Nobel Prize for Chemistry for elucidating the nature of the chemical bond and solving structures of proteins. That he discovered hemoglobin S, the molecular cause of sickle cell anemia, was no less astonishing.

In addition to his brilliant laboratory research, Pauling also had a unique role as a science activist. Well known in the 1950s for his opposition to U.S. nuclear weapons development, Pauling's public visibility caused the U.S. State Department to revoke his passport. Falsely accused of being a communist, Pauling frequently provoked the right wing of American politics during and after the McCarthy era.

Lecturing prodigiously around the world, Pauling often presented technical lectures about chemistry during the day and educational lectures to the public at night. The public lectures focused on the danger of thermonuclear weapons testing, made internationally famous due to publication of his bestseller entitled *No More War* (1). A remarkable raconteur, Pauling conveyed nuclear testing information in terms that all could understand, saying that the dangers that radioactive fallout held for the health of innocent people had become an international crisis. Pauling seemingly used every possible opportunity to speak out.

In April 1962, Pauling was one of 49 Nobel laureates invited to a black tie dinner at the White House hosted by President Kennedy—an event Kennedy later recalled as “the most extraordinary collection of talent, of human knowledge, that has ever been gathered together at the White House, with the possible exception of when Thomas Jefferson dined alone.” Not about to miss an opportunity to publicly press for an end to nuclear testing, Pauling in shirtsleeves joined a protest on the sidewalks around the White House bearing a sign: “Mr. Kennedy, Mr. Macmillan, WE HAVE NO RIGHT TO TEST.”

We viewed this at home on the evening news. Apparently Kennedy was well aware of Pauling's protest and was reported to have smiled when meeting Pauling in the reception line. Known for his wit, Kennedy remarked that he understood that Pauling had been around the White House already (2). But he recognized the significance of Pauling's protests, and just 6 weeks before his assassination, the president signed the Limited Test Ban Treaty, preventing nuclear arms testing in the atmosphere, an event that markedly



reduced the tension of the nuclear arms race. As Kennedy was being buried in Arlington Cemetery, the Pauling family was preparing for his trip to Oslo to receive his second Nobel: the 1962 Peace Prize.

Others have frequently commented on Pauling's exceptional personality. Roughly 9 years ago at a reception in Stockholm, I had the opportunity to meet James Watson. Having reread his classic *The Double Helix* (3) on a family wilderness canoe trip the summer before, I complimented Watson on the first sentence—a grabber that draws the reader's attention: “I have never seen Francis Crick in a modest mood.” Watson nodded when I explained that Linus Pauling had been a family friend, then grinned broadly and added, “I have also never seen Linus Pauling in a modest mood.” To which I thought to myself, “Do heroes really need to be modest?”

Medicine as a Scientific Career

My path to science came not from a pressing ambition to become a prominent researcher but from a desire to pursue a career in medicine. This was in part a cultural emphasis learned early at home. As much as our father emphasized math and science, our mother,

a farm girl who never had the opportunity to attend college, was self-taught through a love of reading. And she read to us every night from the children's Bible as well as great books like the Laura Ingalls Wilder series. I remember snuggling with my siblings on the sofa during cold Minnesota evenings as Mother read.

Although I was just a small child, it became obvious to me that two of my five siblings were already manifesting life-long disabilities: a brother diagnosed with mental retardation and motor skill dysfunction and a sister with a variant of Tourette syndrome and a lack of impulse control. While my parents did not dwell on it excessively, we were nevertheless reminded that all were not equivalently blessed, and we were always encouraged to use our talents for the well-being of those less fortunate.

Also strongly encouraged in our Minnesota Norwegian Lutheran community was a

sense of responsibility for those in the developing world. Medical missionaries were widely respected throughout our community. Minnesota Governor Al Quie's sister, a nurse, and her husband, a surgeon, spent their entire careers attending to the health needs of rural Cameroon. Even our congressman, Walter Judd, had spent a decade as a physician serving the poor in rural China.

Having the opportunity to attend Augsburg College in Minneapolis, where Father taught after leaving St. Olaf, I majored in chemistry and received outstanding lectures and laboratory experiences. Several of my Augsburg classmates were from medical missionary families and had grown up in rural Africa, India, and Asia. This group projected sincere altruism without any hint of self-promotion.

Through an introduction to Richard Varco, a 1955 Lasker Award-winning pioneer in heart surgery, I received the opportunity to undertake summer research at the University of Minnesota Heart Hospital. The experience was pivotal in solidifying my interest in medicine. All five of the summer students I worked with have risen to leadership positions in leading institutions

of academic medicine. Two of my younger brothers must have reached the same conclusion, since both have gone on to become medical doctors.

Accepted at medical school and having finished my college coursework early in the winter of 1970, I had several months for an elective experience. I chose to use the time backpacking throughout east, southeast, and southern Asia and the Middle East. This allowed me the chance to view exotic places but also the opportunity to visit sites of medical research in Thailand and India that intrigued me greatly. Although “going native” often brings unwanted experiences with traveler’s diarrhea and other maladies, I survived the experience excited about the prospect of global health research at Johns Hopkins University, renowned for its international programs.

Early Research Experiences

Probably the most important experience for any young scientist is the opportunity to join an exciting laboratory. Although algorithms may exist for matching students with labs, my experience suggested that personal contact may be much more informative. As a first-year medical student at Johns Hopkins, I became close personal friends with Vann Bennett, a fellow medical student who had been a Stanford wrestler and a big-wave surfer in Hawaii. Vann and I shared a love for vigorous physical exercise, such as bicycle camping trips throughout the Appalachians. Vann’s fascination with biochemistry was something that I grew to admire, and I envied his special bravado—often skipping class assignments to pursue his independent research in a laboratory.

Intrigued by our medical school lectures in microbiology, I became fascinated by the recent advances in understanding the molecular basis of cholera, a horrible diarrheal disease that was sweeping through Asia, killing tens of thousands of infants and small children. I began working on a related project, the molecular basis of the well-known malady *E. coli* traveler’s diarrhea, in the lab of R. Bradley Sack, a young infectious disease researcher at Hopkins. With Brad’s support, I made steady progress, studying the toxin by injecting crude mixtures into the lumen of ligated segments of rabbit small intestine that then became swollen with secreted diarrheal fluid. It soon became clear that purification of the toxin would require more sophisticated technical expertise.

It was through Vann that I came to join the

lab led by Pedro Cuatrecasas, a brilliant Spanish-born physician scientist who had trained at the National Institutes of Health (NIH) with Christian Anfinsen, who had shared the 1972 Nobel Prize in Chemistry for establishing that the primary sequence of a protein, staphylococcal nuclease, determined the structure and catalytic activity of the enzyme.

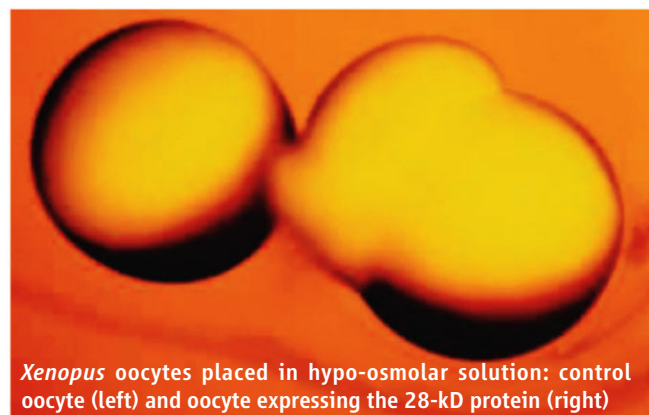
Although only in his 30s, Pedro had already achieved great recognition for pioneering the technique of “affinity chromatography” to biological problems (4). This novel concept used an insoluble matrix of polymeric beads to which small molecules had been covalently attached, and through which complicated biological mixtures such as cytoplasm or solubilized membranes could be filtered. Using affinity chromatography, success had been achieved in isolating, for the first time, receptors for hormones such as insulin, epidermal growth factor, and estrogen. For this work, Pedro was to subsequently share the 1987 Wolf Foundation Prize in Medicine. Despite the fame he achieved from affinity chromatography, Pedro always encouraged us to seek important biological problems, rather than simply seeking problems to apply our technical repertoire to.

After purification of cholera toxin in 1970 and recognition that ganglioside membrane lipids served as receptor sites, Pedro entered the field to characterize the membrane events of cholera toxin activation of membrane adenylate cyclase. This also made the Cuatrecasas lab well suited for studying the elusive *E. coli* enterotoxin. Using ganglioside affinity gels we had prepared, we succeeded in adsorbing the enterotoxin activity to the affinity columns and were able to elute highly enriched subunits of the enterotoxin from the column, allowing us to raise antibodies and develop an immunoassay to diagnose *E. coli* enterotoxic diarrhea from crude stool samples (5).

The prospect of using our new technique to screen individuals in the field in Bangladesh, where diarrheal diseases were rampant, was our long-term objective. As is not uncommon, scaling up a prototype lab test for clinical testing was not simple. Having committed my entire senior year of medical school to the

project, it was still not ready for field testing, so I decided to remain an extra year as a postdoctoral fellow in Pedro’s lab. This was considered highly questionable by the medical student advisors, as well as most of my medical school classmates, since graduating and becoming resident physicians are equivalent to the Holy Grail for medical students.

But for me, remaining associated with the colorful members of Pedro’s team was much more exciting. The group included several that had bypassed lucrative careers in clinical medicine to follow their passions for science. Looking back years later, I think it was clearly the people in Pedro’s lab that made science irresistibly appealing to me: Vann; Gianfredo Puca, a debonair Neopolitan film actor who revolutionized the understanding of femininity by purifying the estrogen receptor; Ignacio Sandoval, a Spanish leftist whose love of biology was only exceeded by his hatred of the Franco regime; Naji Sahyoun, a loyal Palestinian; and Marvin Siegel, the son of an orthodox rabbi, became lifelong best friends after working closely together in the lab. And



Xenopus oocytes placed in hypo-osmolar solution: control oocyte (left) and oocyte expressing the 28-kD protein (right)

even more importantly, I had met my future wife, Mary, who worked in a neurovirology laboratory at Johns Hopkins, and our lives took on a special tone resembling *La Bohème* with a science orientation.

Pathway to a Discovery

The reason one lab makes a discovery where others have failed has been an issue long debated in science. It may well be that each lab is unique in its own way, but this seems overly simplistic, and it may be that a few generalizations are reasonable. So I will summarize, with a little editorializing, how our lab came to discover the protein now known as aquaporin-1 (AQP1), the first defined molecular water channel—using our story to communicate seven “pearls” for younger scientists.

1. Be lucky by keeping your eyes wide open.

After joining the faculty at Johns Hopkins in 1984, my small laboratory was working on human erythrocyte membranes. We set out to isolate the core 32-kD membrane protein of the Rhesus blood group antigens. In addition to the 32-kD Rh, a 28-kD membrane protein copurified. We subsequently realized that the smaller polypeptide was a contaminant and was unrelated to Rh.

2. Consult the wisdom of your colleagues.

The nature of the 28-kD protein was intriguing. Unlike most proteins, the 28-kD polypeptide stained poorly with Coomassie blue, so had not been noticed previously. When it was purified to homogeneity, we realized that the 28-kD membrane protein was extremely abundant and seemed to be a homotetrameric protein, suggesting that it might be a membrane channel. N-terminal protein sequencing provided sufficient information for cDNA cloning that showed that the new protein was related to a series of functionally undefined proteins from diverse organisms, including plants. I spoke with numerous colleagues about the possible role for the 28-kD protein, until one had an insightful idea.

3. Respect but don't overcompartmentalize work and family life. Despite the need to share responsibilities for the raising of children and contributing to domestic duties, it is important to remain flexible. The conversation that helped elucidate the function of the new 28-kD protein occurred at the end of one of our family camping trips. While returning from the Everglades, we stopped in Chapel Hill, North Carolina, so that Mary could visit friends, the children would have a chance to play with their former playmates, and I could drop in on some of my scientific friends. This was to become just one of many examples where our family life catalyzed a scientific advance.

4. Great ideas come when you least expect them. What began as a casual visit with my former professor John Parker, a hematologist and membrane physiologist at the University of North Carolina School of Medicine, became a major event. I shared with Parker what I knew about the 28-kD protein. John quickly recognized that the protein might be something long sought by physiologists: the membrane channel for water. Workers in multiple labs worldwide had



Rural Zambia, 2008

generated indirect evidence supporting the existence of water channels, but none had isolated the putative channel and established its molecular identity.

5. Team up with those bearing needed expertise.

Parker also suggested that I contact Bill Guggino when I returned to Hopkins. Guggino, a membrane-channel physiologist, used techniques in his lab for the expression and study of membrane ion channels. This allowed our postdoctoral fellow Gregory Preston to perform a simple assay demonstrating that frog eggs expressing the new protein became osmotically active and exploded when placed in distilled water (6). This began a long series of collaborations with an expanding group of aquaporin scientists around the world, including renal physiologists at NIH, structural biologists in Basel and Kyoto, microscopists in Aarhus, neuroscientists in Oslo, eye researchers in London and Tokyo, and others. Together, many scientists provided new insight into the fundamental processes involved in renal concentration, vision, skin integrity, brain edema, thermal stress, arsenic clearance, and obesity, as well as the adaptation of plants to drought (7).

6. Don't take yourself too seriously. In the beginning, it was difficult not to take every advance in the aquaporin field personally and view newcomers and com-

petitors with great suspicion. It soon became obvious that we were simply a small section of a new field of science. When the Nobel call eventually came from the Royal Swedish Academy of Science, it was my 78-year-old mother who put it in proper context, "That's very nice, but don't let it go to his head." Her intent was to communicate that awards are nice, but doing something useful for others is really important.

7. Science is about new adventures. The idea of working in global health had been my original career ambition. With the recognition of aquaporins in the parasite causing malaria, we cautiously entered the field. In many ways, this marked a new direction for our lab, but one that we were eager to follow. And with a new direction came a new opportunity: directorship of the Johns Hopkins Malaria Research Institute (JHMRI) at the Bloomberg School of Public Health (8). The new role has been a challenge, but it has been a delight to be part of a program with as much global importance as malaria research. The JHMRI lab programs in Baltimore and fieldwork in rural Zambia are both stimulating and rewarding. And the ease with which malaria crosses borders, killing hundreds of thousands of African children, is a clear reminder of the international aspects of science.

Science as a Tool for International Diplomacy

Science has always been one of the most international of human endeavors, and this trend is certainly increasing. Every year, thousands of young scientists come to the United States from abroad to undertake scientific education and research. Thus, science provides a unique approach to advancing good will toward America in the international arena.

It is no secret that the U.S. government is viewed negatively in the Muslim world, especially after the military intrusions in Iraq and Afghanistan. The Zogby Poll [(9); see table below] reveals a clear bimodal response. The great majority of citizens of five moderate Arab nations held distinctly unfavorable views of the United States in general. In marked contrast, these same individuals held favorable views of U.S. science and technology. This provides an opportunity to use our background as nongovernment scientists to reinforce the positive: that U.S.-generated science and technology may improve the lives of people all around the world.

Country	U.S. in General		U.S. Science and Technology	
	Favorable	Unfavorable	Favorable	Unfavorable
Morocco	11%	88	90	08
Saudi Arabia	04	94	48	51
Jordan	15	78	83	13
Lebanon	20	69	52	46
UAE	14	73	84	12

[From *Impressions of America* (9)]

Having the chance as president of AAAS to participate in some outstanding programs, one stood out as particularly appealing: the AAAS Center for Science Diplomacy. The potential to establish contact and engage with scientists in countries considered adversarial to the U.S. government is an opportunity for science to serve as a unique bridge. Founded in 2008, the center is directed by Vaughan Turekian, an atmospheric geochemist and international policy expert, with special advisor Norman Neureiter, a chemist with extensive policy experience. I was greatly pleased to participate as senior scientist in a series of trips abroad (10).

Recognizing that some of our visits were to countries where there are serious intergovernmental tensions related to a wide range of issues such as proliferation, human rights, and economic openness, each visit was undertaken with an independent nongovernmental organization and with private funding, in most cases from the Richard Lounsbery Founda-



tion. Efforts were made to inform appropriate U.S. authorities of such visits, but it was to be plain in every case that we served as representatives of the U.S. scientific and research community, not the U.S. government.

Cuba. Together with members of the New America Foundation, a trip to Havana, Cuba, was made in November 2009. Our trip, the first AAAS visit since 1997, included a visit to the Finlay Center for Vaccines Research and Production, where we observed the Cuban efforts to prevent infectious diseases such as type B meningococcal meningitis. Endemic before the Revolution, malaria has been eliminated from Cuba, despite a heavy malaria presence in Haiti, just to the east. Cuban efforts to provide universal prenatal health care have succeeded in raising the average life span to 78+ years, equivalent to that in the United States.

The University of Havana generates a large number of science graduates, but laboratory opportunities are limited. Certainly the investment of funds in laboratories to train young scientists could be mutually beneficial to Cuba and the United States. Potential scientific collaborations could be rapidly undertaken once the five-decade political standoff between our governments is resolved.

Democratic People's Republic of Korea (DPRK; North Korea). The AAAS Center for Science Diplomacy worked for more than a year with the U.S. Civilian Research Development Foundation to gain an invitation to visit Pyongyang in December 2009. Located only ~500 miles east of Beijing, Pyongyang exists in a world apart from the hustle and thickly polluted air of the vibrant Chinese capital. Having only a modest power grid, Pyongyang has crystal-

clear air and is impeccably neat but frigid due to a lack of interior heating.

It was obvious from our visit that young North Koreans have great prowess with computer systems—also made clear from ongoing collaboration between Kim Chaek University in Pyongyang and Syracuse University in New York. DPRK agricultural and health sciences were far less advanced and represent areas where the United States could be helpful to the DPRK.

After we had spent 1 week under the watchful eyes and gracious hospitality of the State Academy of Science, our hosts exhibited a warmhearted response when presented with the necktie I had worn during my Nobel lecture in Stockholm.

CREDITS: PETER AGRE





Pyongyang University of Science and Technology, 2009

Emblazoned on the shield of the Johns Hopkins Department of Medicine is the word *Aequanimitas*. The term means “imperturbability.” The tie, bearing this word, was given with our wish that it be worn by the first DPRK scientist who wins a Nobel. Although the political distance between our governments has not decreased in the months after our trip, it should be kept in mind that well-intentioned scientists reside in the DPRK. When the U.S. and DPRK governments finally establish an accord, it is expected that the pursuit of peaceful areas of science may be a bridging mechanism.

Myanmar (Burma). Another country of current difficulty due to its longstanding military junta is Myanmar, a nation of 59 million people with rich natural resources. Together with the U.S. Collection Humanitarian Corps, a visit was organized in April 2010. Of several ministries visited, the Ministry of Health may have been most important, because of the heavy infestation of malaria throughout the countryside that is problematic to neighboring countries. Since our visit, national

elections have been held, and the national leader and 1991 Nobel Peace Prize Laureate Aung Sang Suu Kyi has been released from confinement in her home. Although internationally recognized democracy has not yet returned to Burma, it is possible that continued scientific dialogue may contribute to the transparent and ongoing interactions with the international community that serve as a precondition for any sustainable and effective system of governance.

India. The world’s largest democracy, India and the United States have had longstanding science engagement. It was a privilege to attend the 10th anniversary of the Indo-U.S. Science and Technology Forum in December 2010. This included visits to the National Institute of Malaria Research, other laboratories, and university campuses, and a public lecture on science. Despite India’s many social and economic problems, an air of optimism is readily apparent in India, and science seems a large part of it.

Given our longstanding mutual support, Indian scientists shared observations that

reveal their views of Americans. After my lecture to outstanding high-school science students at the University of Delhi, I was presented with a painting of the beautiful multiarmed Hindu goddess Saraswati, who is widely worshiped throughout India as the bearer of enlightenment. Our international friends can tell us things we in the United States need to hear.

Final Reflection

Science is the medium of our life’s work. Whether we are frustrated or joyful, we always know that each new day in the lab brings an opportunity to make profound advancements in the understanding of nature that may improve the well-being of others.

One of the talented young people from China in our lab always took the optimistic approach, summarized by the Mandarin character for “crisis.” It is actually two characters: wei, meaning “time of danger,” and ji, meaning “time of opportunity.”

For me, the crisis of living in today’s world includes dangers such as increasing microbial drug resistance, the obesity epidemic, environmental damage, the need for sustainable energy supplies, and hostile relations between countries. It seems clear to me that solutions to each will come through the great opportunities provided by science. And with that in mind, I am optimistic about the future.

危机

Wei Ji – Mandarin for “crisis”

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ASSOCIATION AFFAIRS

Nina Fedoroff: 21st-Century Challenges Require Global Focus by Scientists

Nina Fedoroff is starting her new year in Saudi Arabia, focused on some lifelong research and policy passions. She's establishing a new center for desert agriculture at King Abdullah University of Science and Technology, where scientists from many countries and diverse disciplines will tackle one of the most significant challenges of the 21st century: how to feed a planet in the face of diminished arable land and shrinking freshwater supply.

For 3 years as science adviser at the U.S. State Department and USAID, Fedoroff urged researchers to collaborate on "truly global problems that do not respect political boundaries or political positions." As the incoming AAAS president, she will encourage the association to further expand its international engagement and support a future for science that looks much like the work of her new center.

AAAS is "a wonderful interface between science and society, and this is something that is important throughout the world," Fedoroff said in a recent interview. "And it's never been more important for scientists to work together on the big issues confronting the world: food, energy, and water."

Her own experiences—from working closely with molecular biologists in the former Soviet Union to mentoring a Brazilian postdoctoral fellow—have convinced her that science is well suited to bringing nations together in this enterprise.

Science is an evidence-based exploration of nature, where "it doesn't matter what language the science is done in—the criteria of excellence are really the same," she said. "That makes it possible for scientists of many political persuasions and many religious beliefs to talk to each other."

AAAS is already a leader in supporting science diplomacy and brokering international collaborations, Fedoroff noted, citing programs supported by its Center for Science Diplomacy and Center for Science, Technology, and Sustainable Development, among others. And she is happy that AAAS



Incoming president. Nina V. Fedoroff will succeed Alice S. Huang as AAAS president when the association's Annual Meeting closes on 21 February. Huang will begin a 1-year term as AAAS Board Chair.

is a partner of the Global Knowledge Initiative, a nongovernmental organization founded by Fedoroff and others that facilitates international scientific partnerships.

But AAAS can do even more to help "jump the gap between countries that are well advanced in science and those that are not," she said. "Every country seeks to be a knowledge society today, and I think one of the most important things that scientists can do is to participate in making connections with scientists in less-developed countries to provide know-how, collaboration, and sometimes even materials."

Fedoroff received the 2006 National Medal of Science from U.S. President George W. Bush for her pioneering research in the fields of plant genetics, plant responses to environmental stress, and genetically modified crops. She received her Ph.D. in molecular biology from Rockefeller University in 1972. In addition to her role as visiting professor at King Abdullah University, she is an Evan Pugh Professor of Pennsylvania State University and a member of the external faculty of the Santa Fe Institute.

She served on the AAAS Board of Directors from 2000 to 2004 and was elected a AAAS Fellow in 2010.

She will succeed Alice S. Huang as pres-

ident when the AAAS Annual Meeting closes on 21 February; Huang will begin a 1-year term as chairperson of the AAAS Board.

As science adviser to the State Department, Fedoroff was instrumental in bringing more scientists to work in U.S. embassies, overseeing the deployment of the first U.S. science envoys, and developing and maintaining science and technology agreements with many countries.

With the new U.S. Congress in place this month, Fedoroff said it is difficult to predict the fortunes of domestic and international science programs in the near future. Targeted programs in energy and climate change research may not disappear, she suggested, "but I think that the budget for basic science

probably won't expand a whole lot."

"Some of our champions of science are retiring from Congress, and I think it's going to be...even more important for scientists to come to Washington and make themselves heard," particularly on the scientific consensus regarding global climate change, Fedoroff said. "I think they have to come, they have to testify, they have to write, they have to lecture—anything they can do to get the message across to the public."

Fedoroff thinks that researchers especially need to sharpen one message: How scientists do their jobs. They work "by carefully constructing evidence and testing hypotheses," she said, drawing a conclusion only when the weight of the evidence supports that conclusion. People in government or business, she said, "often don't understand that science isn't just another point of view or opinion."

Fedoroff sees the AAAS presidency as another chance in her wide-ranging career to share science's potential. "I remember sitting in my study 20, 25 years ago and wishing that I could have a larger voice," she said. "I'm delighted to have that larger voice, because I think that one's accomplishments, one's 15 minutes of fame, need to be put to good use, and I can't think of a better use than communicating about science."

—Becky Ham

AAAS Members Elected as Fellows

In December 2010, the AAAS Council elected 503 members as Fellows of AAAS. These individuals will be recognized for their contributions to science and technology at the Fellows Forum to be held on 19 February 2011 during the AAAS Annual Meeting in Washington, D.C. Presented by section affiliation, they are:

Section on Agriculture, Food, and Renewable Resources

Marcus Alley, Virginia Tech • Clifton A. Baile, Univ. of Georgia • Jerome F. Baker, Sigma Xi • Warwick M. Bayly, Washington State Univ. • Paul M. Bertsch, Univ. of Kentucky • Bryony C. Bonning, Iowa State Univ. • Kenneth J. Boote, Univ. of Florida • Claude E. Boyd, Auburn Univ. • Ingrid C. Burke, Univ. of Wyoming • Arun K. Chatterjee, Univ. of Missouri-Columbia • Martin B. Dickman, Texas A&M Univ. • Steven Fales, Iowa State Univ. • Bradley W. Fenwick, Univ. of Tennessee, Knoxville • Stanton Gelvin, Purdue Univ. • Scott A. Jackson, Purdue Univ. • Jiming Jiang, Univ. of Wisconsin-Madison • Phyllis E. Johnson, Univ. of North Dakota • William C. Koskinen, USDA-ARS • Robert I. Krieger, Univ. of California, Riverside • Thomas A. Miller, Univ. of California, Riverside • David Neale, Univ. of California, Davis • Yakov Pachepsky, USDA-ARS • John Ryan, International Center for Agricultural Research in the Dry Areas, Syria • Eugene Sander, Univ. of Arizona • Johan Six, Univ. of California, Davis • Laosheng Wu, Univ. of California, Riverside • Scott R. Yates, USDA-ARS • Frank G. Zalom, Univ. of California, Davis • Yong-Guan Zhu, Chinese Academy of Sciences

Section on Anthropology

Richard A. Diehl, Univ. of Alabama • Agustín Fuentes, Univ. of Notre Dame • Richard L. Jantz, Univ. of Tennessee, Knoxville • Michelle Lampl, Emory Univ. • Paul W. Leslie, Univ. of North Carolina, Chapel Hill • Fiona B. Marshall, Washington Univ., St. Louis • Anne C. Stone, Arizona State Univ. • Samuel D. Stout, Ohio State Univ.

Section on Astronomy

Dale P. Cruikshank, NASA Ames Research Center • Wendy Freedman, The Observatories of the Carnegie Institution for Science • Lee W. Hartmann, Univ. of Michigan • Bradley M. Peterson, Ohio State Univ. • Marc Howard Pinsonneault, Ohio State Univ. • Michael Werner, Jet Propulsion Laboratory • Aleksander Wolszczan, Pennsylvania State Univ.

Section on Atmospheric and Hydrospheric Sciences

Susan K. Avery, Woods Hole Oceanographic Institution • Wallace S. Broecker, Columbia Univ. • Scott C. Doney, Woods Hole Oceanographic Institution • Catherine Gautier, Univ. of California, Santa Barbara • Roberto César Izaurralde, Pacific Northwest National Laboratory • Sydney Levitus,

National Oceanic and Atmospheric Administration • Michael Oppenheimer, Princeton Univ. • Claire L. Parkinson, NASA Goddard Space Flight Center • Peter Schlosser, Columbia Univ.

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The Newest Synthesis: Understanding the Interplay of Evolutionary and Ecological Dynamics

Thomas W. Schoener

The effect of ecological change on evolution has long been a focus of scientific research. The reverse—how evolutionary dynamics affect ecological traits—has only recently captured our attention, however, with the realization that evolution can occur over ecological time scales. This newly highlighted causal direction and the implied feedback loop—eco-evolutionary dynamics—is invigorating both ecologists and evolutionists and blurring the distinction between them. Despite some recent relevant studies, the importance of the evolution-to-ecology pathway across systems is still unknown. Only an extensive research effort involving multiple experimental approaches—particularly long-term field experiments—over a variety of ecological communities will provide the answer.

Three-quarters of a century ago, the New Synthesis—an integration of paleontology, systematics, morphology, and genetics—captured the imaginations of evolutionary biologists. Ecology, then less well developed, played a relatively small role in this synthesis, even though the major influence of ecological processes on evolutionary trajectories was recognized. The dynamical effect of evolution on ecology, however, has only recently become widely appreciated. Here I review the emerging field of eco-evolutionary dynamics, whose major precept is that both directions of effect—ecology to evolution and evolution to ecology—are substantial. The general argument is as follows: Many studies have documented that ecological change affects evolution; indeed, natural selection is where ecology and evolution meet. Such studies of “evolution in action,” i.e., over observable time scales, show that evolution can be very rapid. This opens the possibility that evolutionary dynamics can also affect ecology; specifically, evolution can occur so quickly that ecological and evolutionary change may be commensurate in time and may interact in a feedback loop. Despite the potential for a directional influence of evolution on ecology, conceptual bolstering from mathematical theory, and some recent empirical studies, we still don’t know if the evolution-ecology pathway is frequent and strong enough in nature to be broadly important.

Ecology Affects Evolution

Many studies have shown that evolution is shaped by ecology. The most detailed of these concern ongoing observations of natural selection (1) driving phenotypic response to changing environmental conditions. A well-known example is found in work on a Galápagos ground finch, *Geospiza fortis*. In this species, larger beaks dominated the population after dry years when large seeds were more available. Correspondingly, after wet years

the direction of selection reversed, favoring smaller beaks that were more appropriate for handling the small seeds produced in the wet environment. These results demonstrate an immediately observable adaptive response to selection favoring animals with the beak best suited for handling available food (2). A second set of examples involves life histories and morphologies of fish. Intense harvest resulted in Atlantic cod (*Gadus morhua*) evolving earlier maturation and a smaller adult body size (3). A similar pattern was observed among guppies (*Poecilia reticulata*) after experimental predator introduction (4). In these cases, rapid adaptation for smaller body size and an earlier age at reproduction occurred in response

to intense predation. Finally, steelhead and rainbow trout are both members of the species *Oncorhynchus mykiss*; however, steelhead migrate, whereas rainbows do not. In an already classic case not only of adaptation but also of rapid speciation, steelhead trout introduced (in 1910) to a stream isolated above a waterfall evolved a rainbow trout non-migratory life-style. Rainbow-like steelhead later able to move across the falls were reproductively isolated from the ancestral steelhead population (5). In short, here adaptation to local ecological conditions resulted in not only a change in behavior, but also adaptive divergence and subsequent inability to breed with the ancestral population.

Evolution Can Be Fast...

By definition, observations of evolution in action document extremely rapid evolution. The number of reliable studies in which natural selection has been observed has increased markedly since Endler reviewed them in his classic 1986 book, *Natural Selection in the Wild*. The most comprehensive discussions are by Reznick and colleagues (4, 6), who found 47 studies demonstrating or implying rapid evolution for a variety of traits (morphological, physiological, life-history, phenological, and behavioral). Most examples involve colonization events, i.e., invasion, or local changes in semi-isolated populations across a varying environment (7). Although the examples are all from nature (rather than the laboratory), humans, by deliberate or accidental introduction or other environmental modification, have spurred many of them: Human predatory activities are especially effective (8). Indeed, without recent and severe anthropogenic actions, the list

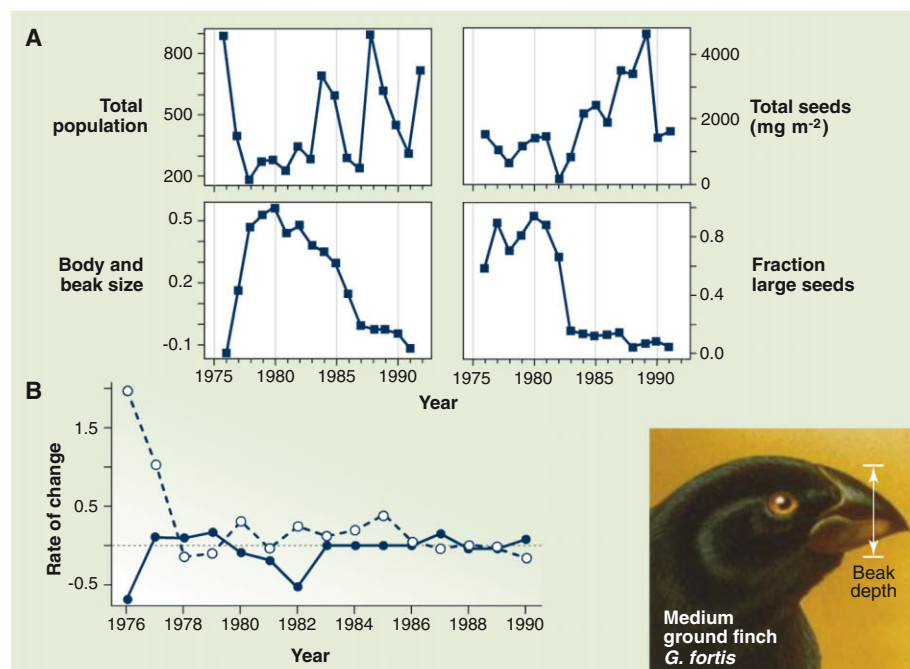


Fig. 1. (A) Dynamics for evolutionary and ecological traits in *G. fortis*. **(B)** The annual ecological (solid line) and evolutionary (dashed line) contributions to the total rate of change (per year) in population growth rate. [From Hairston *et al.* (11)]

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of cases of rapid evolution would be much shorter. In view of such studies, our concept of evolutionary time is also changing rapidly. As Fussmann *et al.* (9) highlight: “By rapid evolution we mean evolution occurring on time scales tractable in laboratory studies of population dynamics—up to about 1000 generations but typically many fewer.” This was much shorter than the half-million years for evolutionary time given by the ecologist Slobodkin in his classic 1961 book, *Growth and Regulation of Animal Populations* (10); his ecological time was “on the order of ten times the generation time of the species involved.” Hairston *et al.* (11) go the farthest in defining “rapid evolution” as “a genetic change rapid enough to have a measurable impact on simultaneous ecological change,” thus coming full circle.

These reinterpretations of how quickly evolution can occur open the door to the possibility that evolutionary dynamics can affect ecological dynamics—in principle. We have just seen that, because evolution can be so fast (4, 6, 8), evolutionary and ecological time can be commensurate. Indeed, natural selection and population dynamics are both affected by births and deaths of individuals (12–14). In fact, in the founding paper on selection gradients (15), mortality in house sparrows (*Passer domesticus*) after a winter storm provided an illustrative example for the computations. To paraphrase Kokko and López-Sepulcre (16): Assessing fitness is ultimately counting offspring that transmit genes to future generations. Therefore, population dynamics (an ecological entity) can depend on the fitness of population members (an evolutionary entity). Similarly, an individual’s fitness can depend on the ecological trait of population density, including densities of conspecifics, heterospecific competitors, prey, or predators. Although tightly related, however, the link between natural selection and population dynamics is not entirely straightforward. Specifically, the measure of fitness used when calculating selection [e.g., (15)] is relative to other members of the population (i.e., how many more offspring does individual A produce relative to individual B, or the rest of the population). In contrast, in population dynamics we are concerned with the absolute output of an individual and how that contributes to population properties.

As summarized, that ecology affects evolution is the cornerstone of the natural selection concept. But can the reverse also happen to a substantial extent? More precisely, what is the contribution to population growth of evolutionary factors, such as genetically based phenotypic change, relative to ecological factors such as changes in the predation or resource regime or abiotic environmental change? The seminal 2005 paper by Hairston *et al.* (11), in which what has come to be called the “Geber method” was introduced, first tackled this question. Using a clever technique based on analysis of variance, Hairston *et al.* partitioned the factors causing year-to-year variation in population growth rate into, roughly speaking, evolutionary and ecological components. In the finch *G. fortis* discussed above (2), evolutionary contributions (via beak shape

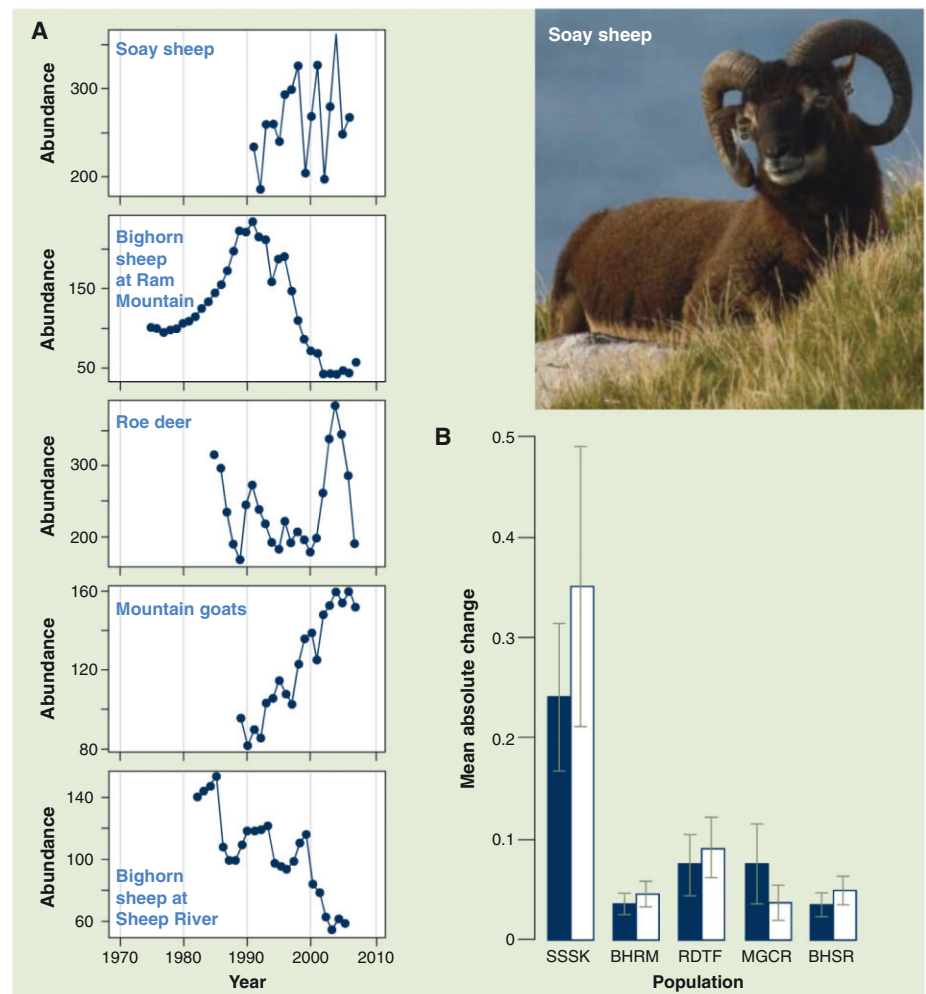


Fig. 2. (A) Population numbers for the five ungulate species in Ezard *et al.*’s (18) study. Time series are shown for the periods of each population analyzed. Species are (from top to bottom) Soay sheep, bighorn sheep at Ram Mountain, roe deer, mountain goats, and bighorn sheep at Sheep River. **(B)** Mean absolute change of environment (blue bars) and phenotype (white bars) with 95% parametric confidence intervals, calculated following Hairston *et al.* (11). The change quantifies the effects on population growth of environmental and phenotypic changes, respectively. Study populations follow the order in the left panel.

and body size) exceeded ecological contributions (via seed density and fraction of large seeds) by a factor of 2.2 (Fig. 1). For Hairston *et al.*’s data on the copepod *Onychodaptomus sanguineus*, the evolutionary contribution (via life history: whether diapausing or immediately hatching eggs are produced) was one-fourth the ecological contribution (via fish predation), which they call “less than in the finch example, but nevertheless substantial.” They also applied a related method to Abrams and Matsuda’s (17) model of predator-prey dynamics, showing that evolutionary effects are substantial but not dominant, being 63% of ecological effects. Ezard *et al.* (18) recently used the Hairston *et al.* methodology to partition population change for five ungulate species. They characterize the evolutionary (morphological variables) versus ecological (vegetational and climatic variables) contributions as “statistically indistinguishable” (Fig. 2). Indeed, the best supported model includes both evolutionary and ecological factors, as well as the interaction between them.

In retrospect, we can ask why the correspondence of ecological and evolutionary time was not recognized [e.g., (10)]. Kinnison and Hairston (19) suggest two major reasons. First, fitness gains made in the course of selection might not much influence the equilibrium population size, e.g., the carrying capacity, because of strong negative density-dependent regulation. Saccheri and Hanski (20) conclude that explicit demonstrations of this are few, despite theoretical plausibility. Second, the rate of evolution in the paleontological record is “ponderous,” and this is inconsistent with rapid evolution observed in contemporary studies. Thompson (21) suggests this is because episodes of one-way directional selection are interspersed with episodes of stasis or even episodes of selection in the other direction. Hendry and Kinnison (22), building on Gingerich (23), combined data from various studies and showed that the longer the observation period, the weaker the evolution—thus a kind of bias exists [the same trend was found later by Hoekstra *et al.* (24) for studies of

selection measured by survival]. To explore the issue farther, Hairston *et al.* (11) supplemented Hendry and Kinnison's data with per-generation data for two species—the finch and the copepod discussed above—and found that short time intervals could give very low rates of evolution as well as high ones [Fig. 3; see also (6, 25)]. So there is a bias, but it is mainly that the variance of the rates decreases with length of the observation period. Further, that selection can frequently change direction has recently become well established thanks to Siepielski *et al.*'s (26) review of 89 studies incorporating 5519 estimates of selection.

As a result of these and other studies, a frequent correspondence of ecological and evolutionary time is now widely accepted. For example, Carroll *et al.* (27) say “the real distinction between microevolution [short-term] and macroevolution [long-term] may lie only in the degree to which the factors causing evolution are fluctuating or are gradually and persistently directional, and not in the ecological significance of that evolution.” Indeed, the entire issue has been raised almost to the level of a scientific manifesto, where opposition is considered misguided and fruitless. Thus, Kinnison and Hairston (19) could say: “The notion that evolutionary processes are vanishingly slow, and that contemporary evolution is exceptional, does not match current scientific knowledge and is counterproductive.”

...But Few Empirical Examples Exist of Evolution Affecting Ecological Dynamics

Four recent reviews (28–31) have shown that genetic variation within a species can affect its ecological community. It is now even becoming possible to generalize about what ecological units are most affected: Bailey *et al.* (30) showed that effects of within-plant-species genetic variation are strongest at the individual level (phytochemistry, physiology, morphology) and weakest at the community (species richness, total abundance, community composition) and ecosystem (carbon accumulation, productivity, soil-nutrient dynamics) levels.

These reviews generally deal with static properties, or what Losos (32) has called “retrospective studies, how present day ecological processes can be understood as the outcome of historical events.” In contrast, to understand the ongoing effects of evolution on population dynamics, we need studies over multiple generations in real time. How many might there be? An attempt to determine this number precisely was made in 2007 by Fussmann *et al.* (9), who isolated “the handful of [studies] that... come close to providing empirical support for eco-evolutionary community dynamics.” Their criteria were as follows: (i) Does the study document change of abundance of multiple populations over several generations? (ii) Is there a record of genetic frequencies and their changes over time? (iii) Is there a plausible mechanistic link between ecological and evolutionary dynamics? (iv) Is there a control reporting ecological dynamics in the absence of evolution? No single study fulfilled all criteria, and only eight studies came close to qualifying. Of

these, all satisfy criterion 1, but five fail at criterion 2 or 4, and three meet neither (33). Six studies were performed in the laboratory and two in the field (the latter were observational, not experimental, and include the finch study discussed above).

Is the lack of field-experimental studies a deficiency? Two properties of field experiments argue that it is. First, field conditions are natural, incorporating most of the background of the phenomena we are trying to understand. Second, experimental design is rigorous in that it employs

used combinations of guppy and killifish (*Rivulus hartii*) populations with different evolutionary histories to simulate invasion, one-species evolution, and coevolution. They found responses in ecosystem properties such as algal biomass and decomposition. In some cases, the evolutionary treatments had larger effects than the ecological (invasion) treatment. Bassar *et al.* (37), also using guppies, showed that different phenotypes were associated with different values of ecosystem structures (biomasses of various groups) and functions (productivity, nutrient flux, leaf-decomposition rates), much as Harmon *et al.* showed. Although all experimental and field [or mesocosm (38)] based, none of these studies was dynamic in Fussmann *et al.*'s sense (with the partial exception of Johnson *et al.*, which ran for 2 years). Rather, most fall into Losos's (32) “retrospective” category—how ecology can result from historical events.

Nine studies appearing after Fussmann *et al.*'s review went to press are multigenerational. Three were field-observational and on mammals: Pelletier *et al.* (39) on Soay sheep, Ozgul *et al.* (40) on yellow-bellied marmots (*Marmota flaviventris*), and Ezard *et al.*'s ungulate study (18) discussed above. The six others were experimental but were conducted in the laboratory: Kerr *et al.* (41), Bull *et al.* (42), and Brockhurst *et al.* (43) on bacteria and phage; Lennon and Martiny (44) on cyanobacteria and viruses; terHorst *et al.* (45) on protozoans and mosquitoes; and Becks *et al.* (46) on algae and rotifers. To elaborate on one, Kerr *et al.* devised a metapopulation using plates on which the bacterial host *Escherichia coli* inhabited media-filled wells. The pathogenic phage always wiped out the bacteria within each well

and then went extinct, resulting in an empty well that could be recolonized by bacteria. The setup selected over time for “prudent” phage if migration between wells was restricted, whereas “rapacious” phage was selected under unrestricted migration. The evolution of the prudent variety is reminiscent of *Myxoma* virus and rabbits, one of Fussmann *et al.*'s qualifying examples: Less virulent virus strains were selected for. These laboratory studies provide valuable insights into the mechanics of the eco-evolutionary feedback process, and the observational studies analyze long-term field data in fundamentally new ways. Still, no field-experimental study has yet been published. An example of the form such a study can take is given in Fig. 4, which sketches an ongoing study using two species of island lizards.

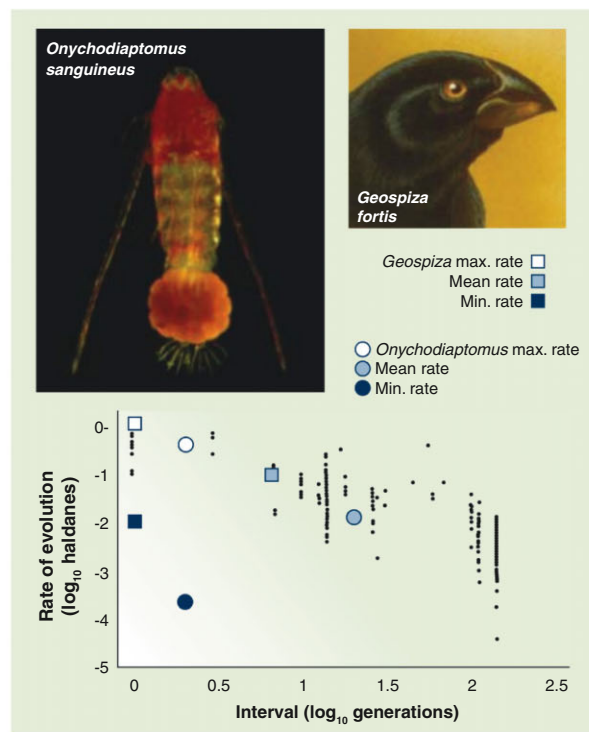


Fig. 3. Relation between rate of phenotypic evolution and number of generations over which measurements were made. Original figure (small dots) is from Hendry and Kinnison (22) with new calculations added for the finch *G. fortis* and the copepod *O. sanguineus*, showing the slowest, most rapid, and the average rates of evolution (per generation) over the 30- and 10-year, respectively, periods of study. [From Hairston *et al.* (11)]

random selection of arenas for the various treatments. Four field-experimental studies of how evolution affects ecological properties have appeared since the review of Fussmann *et al.*—would any meet their criteria? Harmon *et al.* (34) showed that combinations of two species of sticklebacks *Gasterosteus aculeatus* (from different habitats and with unique morphologies that had evolved repeatedly from a common ancestor) differentially affected community-ecological properties (primary production, dissolved organic matter, prey species diversity). Johnson *et al.* (35) experimentally generated selection on biomass, life history, and herbivore resistance of the primrose, *Oenothera biennis*. The resulting evolutionary changes affected abundance and diversity of the associated arthropods. Palkovacs *et al.* (36)

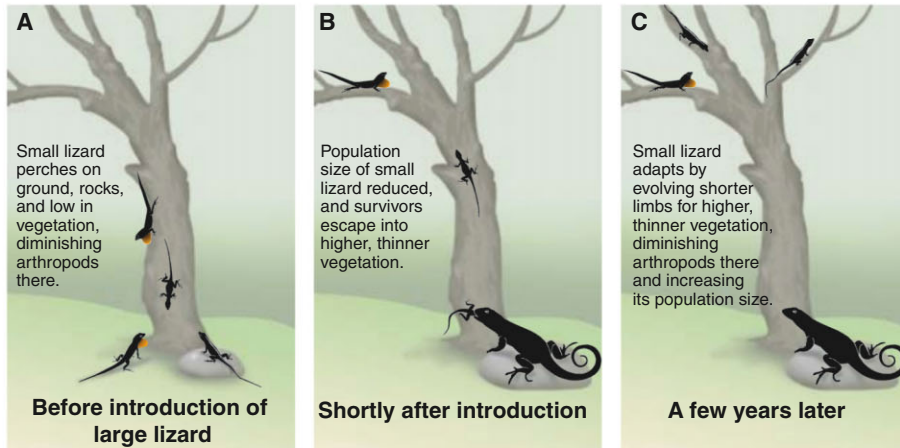


Fig. 4. Experimental approach used in ongoing study of eco-evolutionary dynamics in Caribbean lizards. **(A)** Before introduction of the large predatory lizard *Leiocephalus carinatus* (curly-tailed lizard), the smaller *Anolis sagrei* is found both on the ground and trunks of trees. **(B)** Introduction of predators may result in reduced population size and change in habitat use by *A. sagrei*. **(C)** Rapid evolution of *A. sagrei* could be precipitated by change in selection pressures, and this may have amplifying effects on the system in the form of altered arthropod densities and distributions, as well as increased population size of *A. sagrei* [as argued in Strauss *et al.* (54)].

Feedback Loops Between Evolution and Ecology: Will They Be Important or Trivial?

Dobzhansky notoriously said in 1964: “Nothing in biology makes sense except in the light of evolution.” This was supplanted half a century later by Grant and Grant’s (2): “Nothing in evolutionary biology makes sense except in the light of ecology.” Pelletier *et al.* (12) quickly followed with “Nothing in evolution or ecology makes sense except in the light of the other,” and this sentiment is pretty much where we are today. If ecology affects evolution (long supported) and evolution affects ecology (becoming increasingly supported), then what? The transformed ecology might affect evolution, and so on, back and forth in a feedback loop. Kokko and López-Sepulcre (16) call this “ecogenetic feedback”: “If density influences everyone’s reproductive prospects to the same extent, one has merely restated the ecological concept of density dependence. But if density variation has a differential effect on individual fitness depending on...phenotype, we have a feedback loop. In this loop, individual behavior or life history, influenced by genes, has an effect on population dynamics...and the resulting change in population dynamics in turn...[may] differentially favour...[certain] genotypes...in the population...”

The idea of feedback between evolution and ecology is not itself new. One of Fussmann *et al.*’s examples, Pimentel (47), developed his genetic feedback concept and tested it in the lab half a century ago. Taper and Case’s 1985 character displacement theory (48) modeled coevolution of competing species’ phenotypes and food niches while allowing food resources to change dynamically. Chitty also used a feedback idea to explain cycling selection between dispersers and homebodies in rodents (49); however, little supporting evidence was found and explicit modeling gave the opposite result—natural selection diminished cyclical behavior (50, 51).

Given this long history, what then is new about the emerging field of eco-evolutionary dynamics? Palkovacs *et al.* (36) say: “it remains a frontier to experimentally examine the ecosystem effects of dynamically evolving (and coevolving) populations in the wild...one potentially critical element that can only be captured using dynamic experiments is the eco-evolutionary feedback...” Herein lies the novelty current researchers are exploring. Because our investigations to reveal the role of such feedback are just beginning, it is hard to guess at the outcome. There are large open questions, the most important of which, according to Thompson (21), “is whether the persistence of interactions and the stability of communities truly rely upon ongoing rapid evolution...or whether such rapid evolution is ecologically trivial.” It is a question well worthy of the large research effort it will take to learn the answer.

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Rapid Pneumococcal Evolution in Response to Clinical Interventions

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Epidemiological studies of the naturally transformable bacterial pathogen *Streptococcus pneumoniae* have previously been confounded by high rates of recombination. Sequencing 240 isolates of the PMEN1 (Spain^{23F}-1) multidrug-resistant lineage enabled base substitutions to be distinguished from polymorphisms arising through horizontal sequence transfer. More than 700 recombinations were detected, with genes encoding major antigens frequently affected. Among these were 10 capsule-switching events, one of which accompanied a population shift as vaccine-escape serotype 19A isolates emerged in the USA after the introduction of the conjugate polysaccharide vaccine. The evolution of resistance to fluoroquinolones, rifampicin, and macrolides was observed to occur on multiple occasions. This study details how genomic plasticity within lineages of recombinogenic bacteria can permit adaptation to clinical interventions over remarkably short time scales.

Streptococcus pneumoniae is a highly recombinogenic human nasopharyngeal commensal and respiratory pathogen estimated to be responsible for a global burden of almost 15 million cases of invasive disease in 2000 (1). Since the 1970s, the susceptibility of the pneumococcal population to antibiotics has decreased, largely as a consequence of the emergence and spread of a few multidrug-resistant clones (2).

The first recognized example was Pneumococcal Molecular Epidemiology Network clone 1 (PMEN1), an *S. pneumoniae* lineage typically identified as being sequence type (ST) 81 and serotype 23F, as well as exhibiting resistance to multiple antibiotics, including penicillin. The genome sequence of the first identified member of the clone, isolated in a hospital in Barcelona in 1984, revealed that it had acquired a Tn5252-type integrative and conjugative element (ICE) that carries a linearized chloramphenicol resistance plasmid and a Tn916-type element with a *tetM* tetracycline-resistance gene (3).

This lineage was subsequently found to be present in Africa, Asia, and America (4–8) and, by the late 1990s, was estimated to be causing almost 40% of penicillin-resistant pneumococcal disease in the USA (9). Following the introduction of a heptavalent conjugate polysaccharide vaccine (PCV7) in many countries since 2000, which includes capsule type 23F as one of its seven antigens, a decrease in the frequency of serotype 23F invasive disease and carriage has been observed (10). However, this has been accompanied

by a rise in disease caused by nonvaccine-serotype pneumococci, such as the multidrug-resistant serotype 19A strains now common in the USA (11). Based on evidence from multilocus sequence typing (MLST) from America (12) and Europe (13), some of these are thought to include capsule switch variants of PMEN1 lineage.

To study how this lineage has evolved as it has spread, we used Illumina sequencing of multiplexed genomic DNA libraries to characterize a global collection of 240 PMEN1 strains isolated between 1984 and 2008. Strains were identified either by using MLST or on the basis of serotype, drug-resistance profile, and targeted polymerase chain reaction (14). Selected isolates were distributed among Europe (seven countries, 81 strains); South Africa (37 strains); America (six countries, 54 strains); and Asia (eight countries, 68 strains) (table S1) and included a variety of drug-resistance profiles, as well as five serotypes distinct from the ancestral 23F: 19F (also included in PCV7), 19A, 6A, 15B, and 3.

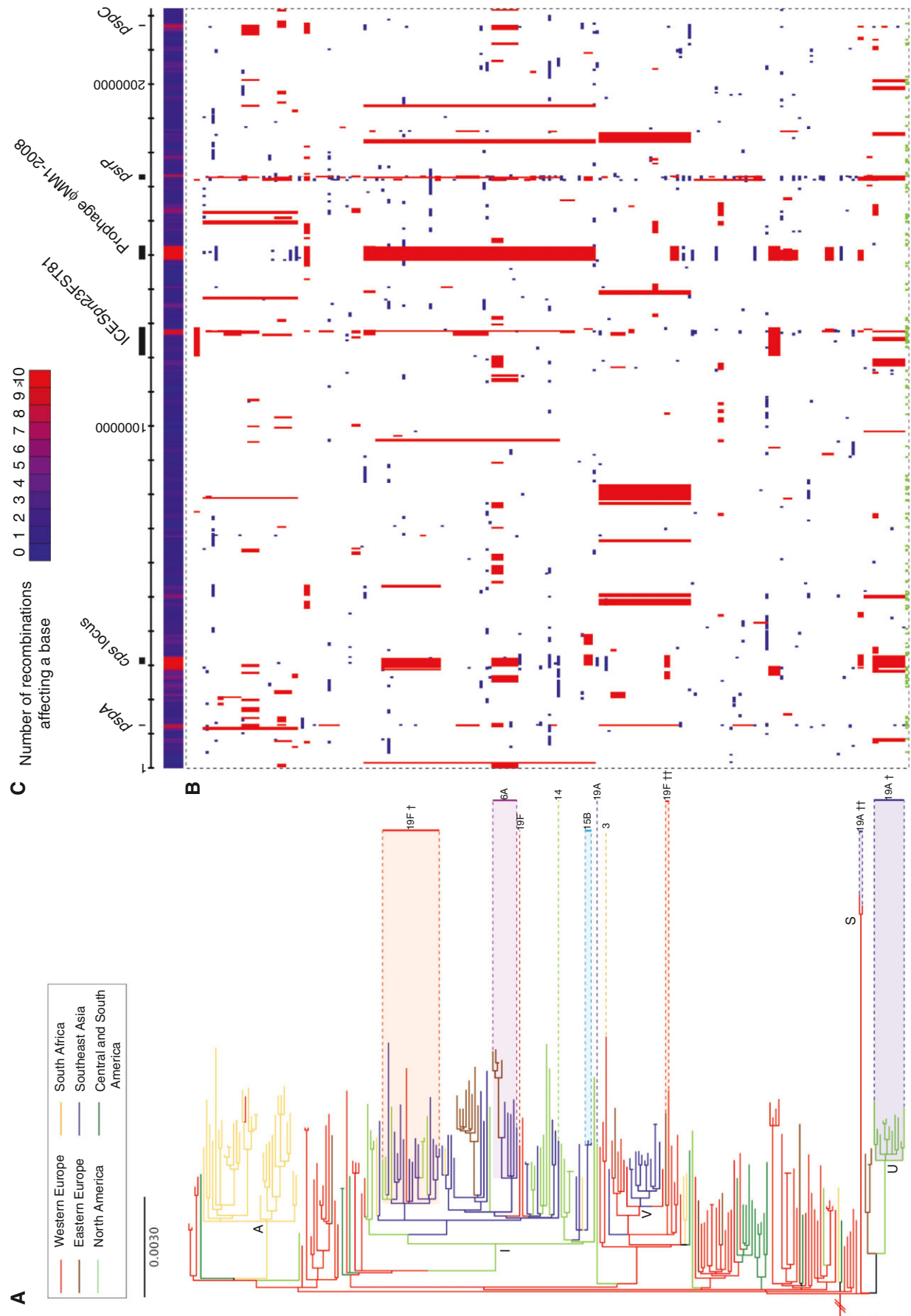
Construction of the phylogeny. Sequence reads were mapped against the complete reference chromosome of *S. pneumoniae* ATCC 700669 (3) and, by using the criteria described in Harris *et al.* (15), 39,107 polymorphic sites were identified within the PMEN1 lineage. Maximum likelihood analysis produced a phylogeny with a high proportion of homoplastic sites (23%) and a weak correlation between the date of a strain's isolation and its distance from the root of the tree (Pearson correlation, $N = 222$, $R^2 = 0.05$, $p = 0.001$) (fig. S1), which suggested that variation was primarily arising through incorporation of imported DNA and not through steady accumulation of base substitutions. As these strains are closely related, sequences acquired by recombination could be identified as loci with a high density of polymorphisms. These events were reconstructed onto the phylogeny and, by using an iterative algorithm (14), an alignment and tree based on vertically inherited base substitutions alone was generated.

From this analysis (Fig. 1), a total of 57,736 single-nucleotide polymorphisms (SNPs) were identified, 50,720 (88%) of which were introduced by 702 recombination events. This gives a

Fig. 1. Phylogeography and sequence variation of PMEN1. (A) Global phylogeny of PMEN1. The maximum likelihood tree, constructed using substitutions outside of recombination events, is colored according to location, as reconstructed through the phylogeny by using parsimony. Shaded boxes and dashed lines indicate isolates that have switched capsule type from the ancestral 23F serotype. †Independent switches to the same serotype are distinguished by annotation with daggers. Specific clades referred to in the text are marked on the tree: A (South Africa), I (International), V (Vietnam), S (Spain 19A), and U (USA 19A). (B) Recombinations detected in PMEN1. The panel shows the chromosomal locations of the putative recombination events detected in each terminal taxon. Red blocks are recombinations predicted to have occurred on an internal branch and, therefore, are shared by multiple isolates through common descent. Blue blocks are recombinations predicted to occur on terminal branches and hence are present in only one strain. The green blocks indicate recombinations predicted to have occurred along the branch to the outgroup (*S. pneumoniae* BM4200), used to root the tree. (C) Biological relevance of recombination. The heat map shows the density of independent recombination events within PMEN1 in relation to the annotation of the reference genome. All regions that have undergone 10 or more recombination events are marked and annotated (Tn916 is encompassed within ICESpn23F5T81).

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per site r/m ratio (the relative likelihood that a polymorphism was introduced through recombination rather than point mutation) of 7.2, less than the previously calculated value of ~ 66 from MLST data (16). By removing recombination events from the phylogeny, the number of homoplasic sites is reduced by 97%, and the tree has significantly shortened branches, such that root-to-tip distance more strongly correlates with date of isolation ($R^2 = 0.46$, $p = < 2.2 \times 10^{-16}$; figs. S1 and S2). The rate at which base substitutions occur outside of recombinations suggests a mutation rate of 1.57×10^{-6} substitutions per site per year (95% confidence interval 1.34 to 1.79×10^{-6}), close to the estimate of 3.3×10^{-6} substitutions per site per year from *Staphylococcus aureus* ST239 (15) and much higher than that found between more distantly related isolates (17). Furthermore, by excluding SNPs introduced through recombinations, the date of origin of the lineage implied by the tree moved from about 1930—which predates the introduction of penicillin, chloramphenicol, and tetracycline—to about 1970 (fig. S1).

Widespread recombination and antigenic variation. Even in this sample of a single lineage, 74% of the reference genome length has undergone recombination in at least one isolate, with a mean of 74,097 base pairs (bp) of sequence affected by recombination in each strain. This encompasses both site-specific integrations of prophage and conjugative elements and homologous recombinations mediated by the competence system. The 615 recombinations outside of the prophage and ICE vary in size from 3 bp to 72,038 bp, with a mean of 6.3 kb (fig. S3). Within these homologous recombinations, there is a distinct heterogeneity in the density of polymorphisms, although it is unclear whether this represents a consequence of the mechanism by which horizontally acquired DNA is incorporated or a property of the donor sequence.

Recombination hotspots are evident in the genome where horizontal sequence transfers are detected abnormally frequently (Fig. 1). One of

the most noticeable is within Tn916, concentrated around the *tetM* gene. Excepting the prophage, the other loci—*pspA*, *pspC*, *psrP*, and the capsule biosynthesis (*cps*) locus—are all major surface structures. PspA and PspC are potential protein vaccine targets implicated in pneumococcal pathogenesis that are targeted by antibodies produced during experimental human carriage studies (18). PsrP is a large ~ 4500 amino acid serine-rich protein, present in a subset of pneumococci, that is likely to be modified by a number of glycosyltransferases that are encoded on the same genomic island. In a mouse model of infection, PsrP-targeting antibodies can block pneumococcal infection (19). Hence, it seems likely that these loci are under diversifying selection driven by the human immune system, and consequently, the apparent increase in the frequency of recombination in these regions is due to the selective advantage that is offered by the divergent sequence introduced by such recombination events.

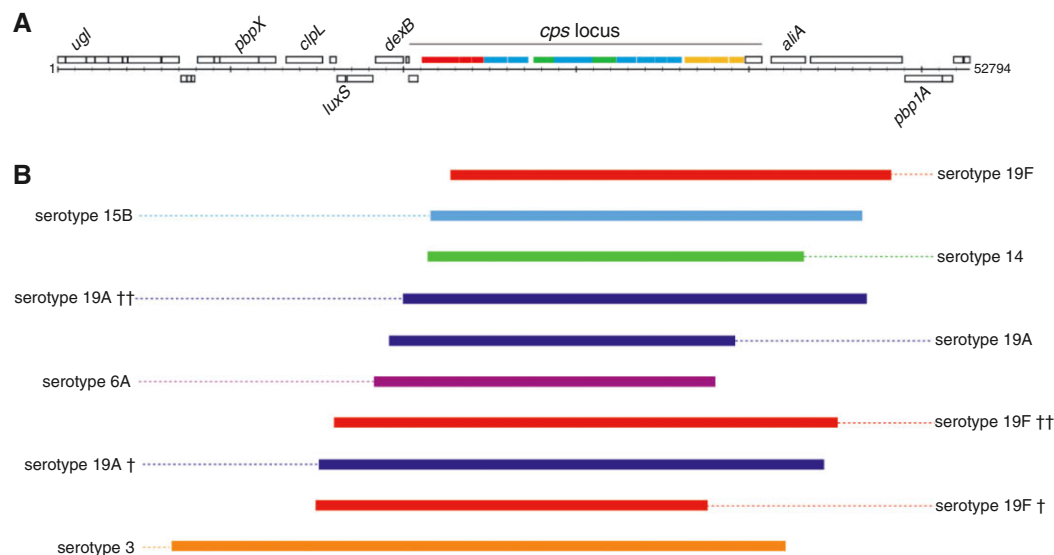
In addition to base substitutions, 1032 small (< 6 -bp) insertion and deletion events can be reconstructed onto the phylogeny, of which 61% are concentrated in the 13% of the genome that does not encode for protein-coding sequences (CDSs), probably because of selection against the introduction of frameshift mutations. Throughout the phylogeny, 331 CDSs are predicted to be affected by either frameshift or premature stop codon mutations. Modeling these disruptive events as a Poisson distributed process occurring at a rate proportional to the length of the CDS, 11 CDSs were significantly enriched for disruptive mutations after correction for multiple testing (table S5). These included *pspA* and a glycosyltransferase posited to act on *psrP* (SPN23F17730). This again suggests there may be a selective pressure acting either to remove (*pspA*) or alter (*psrP*) two major surface antigens. Furthermore, the longest recombination in the data set spans, and deletes, the *psrP*-encoding island, which shows that such nonessential antigens can be quickly removed from the chromosome. These data imply

that the pneumococcal population is likely to be able to respond very rapidly to the introduction of some of the protein antigen-based pneumococcal vaccines currently under development.

Population and serotype dynamics. The spread of PMEN1 can be tracked by using the phylogeography indicated by the tree (Fig. 1). There are several European clades with their base near the root of the tree, and a parsimony-based reconstruction of location supports a European origin for the lineage. Interspersed among the European isolates are samples from Central and South America, which may represent an early transmission from Spain, where the clone was first isolated, to Latin America, a route previously suggested to occur by data from *S. aureus* (15). One clade (labeled A in Fig. 1), containing South African isolates from 1989 to 2006, appears to have originated from a single highly successful intercontinental transmission event. There is also a cluster of isolates from Ho Chi Minh City (labeled V), representing a transmission to Southeast (SE) Asia. However, the predominant clade found outside of Europe (labeled I) appears to have spread quite freely throughout North America, SE Asia, and Eastern Europe, which implies that there are few barriers to intercontinental transmission of *S. pneumoniae* between these regions.

The final non-European group consists of serotype 19A U.S. isolates (labeled U). These all date from between 2005 and 2007 and are distinct from all other U.S. PMEN1 isolates, which have capsular types included in PCV7 (fig. S4). This is evidence of a shift in the PMEN1 population in the USA: Rather than a change in capsule type occurring among the resident population, it seems that it has been eliminated by the vaccine and replaced by a different subpopulation within the lineage that has expanded to fill the vacated niche. Similarly, a pair of Spanish isolates from 2001 (labeled S), the year in which PCV7 was introduced in Spain, that have independently acquired a 19A capsule are not closely associated with any

Fig. 2. Recombinations causing serotype-switching events. **(A)** The annotated *cps* locus of the reference strain. CDSs involved in capsule biosynthesis are colored according to their role. Genes in red are regulatory, those in blue synthesize and modify the oligosaccharide subunits, those in green are involved in polymerization and transport, and those in orange are required for the synthesis of rhamnose. **(B)** Below are delineated the recombinations leading to changes in serotype, colored according to the serotype of the sequence donor. The different events are labeled to correspond with Fig. 1.



other European isolates. The estimated times of origin for clades U (1996; 95% credible interval 1992–1999) and S (1998; 95% credible interval 1996–1999) both predate the introduction of PCV7, and accordingly a third 19A switch, from Canada, was isolated in 1994. Hence, it appears that these changes in serotype after vaccine introduction result from an expansion of preexisting capsular variants, which were relatively uncommon and not part of the predominant population, and would have therefore been difficult to detect before the existence of the selection pressure exerted by the vaccine.

Seven further serotype-switching events can be detected in the data (Fig. 2), including three switches to serotype 19F. The polyphyletic nature of these 19F isolates is supported by the variation observed between the acquired *cps* loci, as is also the case for the 19A isolates (fig. S5). The previously known switches to serotypes 3, 6A, and 15B are only found to occur once each in the phylogeny, and in addition, a single Korean sample that had not been typed was identified as a serotype 14 variant by mapping reads to known *cps* loci (20). The recombination events leading to these switches ranged from 21,780 bp to 39,182 bp in size, with a mean of 28.2 kb. Only 35 homologous recombinations of an equivalent size or larger occur elsewhere in the genome; most such events are much smaller (fig. S3),

which makes it surprising that serotype switching occurs with such frequency and which indicates a role for balancing selection at this locus. Additionally, the span of these events appears to be limited by the flanking penicillin-binding protein genes, the sequences of which are crucial in determining β -lactam resistance in pneumococci (21). Only the recombination causing the switch to serotype 3 affects one of these, and it introduces just a single SNP into the *pbpX* CDS, which does not appear to compromise the strain's penicillin resistance (table S1). Hence, the positioning of these two genes may hinder the transfer of capsule biosynthesis operons from penicillin-sensitive to penicillin-resistant pneumococci via larger recombinations, although size constraints alone could also cause such a distribution.

Resistance to non- β -lactam antibiotics. The strong selection pressures exerted by antibiotics on the PMEN1 lineage are manifest as multiple examples of geographically disparate isolates converging on common resistance mechanisms. Single base substitutions causing reduced susceptibility to some classes of antibiotics have occurred multiple times throughout the phylogeny, as observed in *S. aureus* (15) and *Salmonella* Typhi (22) populations, including mutations in *parC*, *parE*, and *gyrA*, which cause increased resistance to fluoroquinolone antibiotics (23), and changes in *rpoB* causing resistance to rifampicin

(24). The S79F, S79Y, and D83N mutations (25) in *parC* are estimated to occur nine, three, and five times, respectively, in PMEN1; additionally, D435N in the adjacent *parE* gene is found to happen three times. The S81F and S81Y substitutions, in the same position of *gyrA*, are found four and two times, respectively. None of these mutations are predicted to have been introduced by recombination, whereas changes at position H499 of *rpoB* causing rifampicin resistance are introduced twice by horizontal transfer and three times by means of base substitution.

Resistance to macrolide antibiotics tends not to derive from SNPs, but from acquisition of CDSs facilitating one of the two common resistance mechanisms: methylation of the target ribosomal RNA by *erm* genes and removal of the drug from the cell by the macrolide efflux (*mef*)-type efflux pumps. Both can be found in the PMEN1 population, and in all cases, the genes appear to be integrated into the Tn916 transposon (Fig. 3). They are carried by three different elements. Tn917, consisting of an *ermB* gene with an associated transposon and resolvase, inserts into open reading frame *orf9* of Tn916 (26). A second has been characterized as the macrolide efflux genetic assembly (mega) element (27), which carries a *mef/mel* efflux pump system and, in PMEN1, inserts upstream of *orf9*. A third element (henceforth referred to as an omega element, for omega and multidrug-resistance

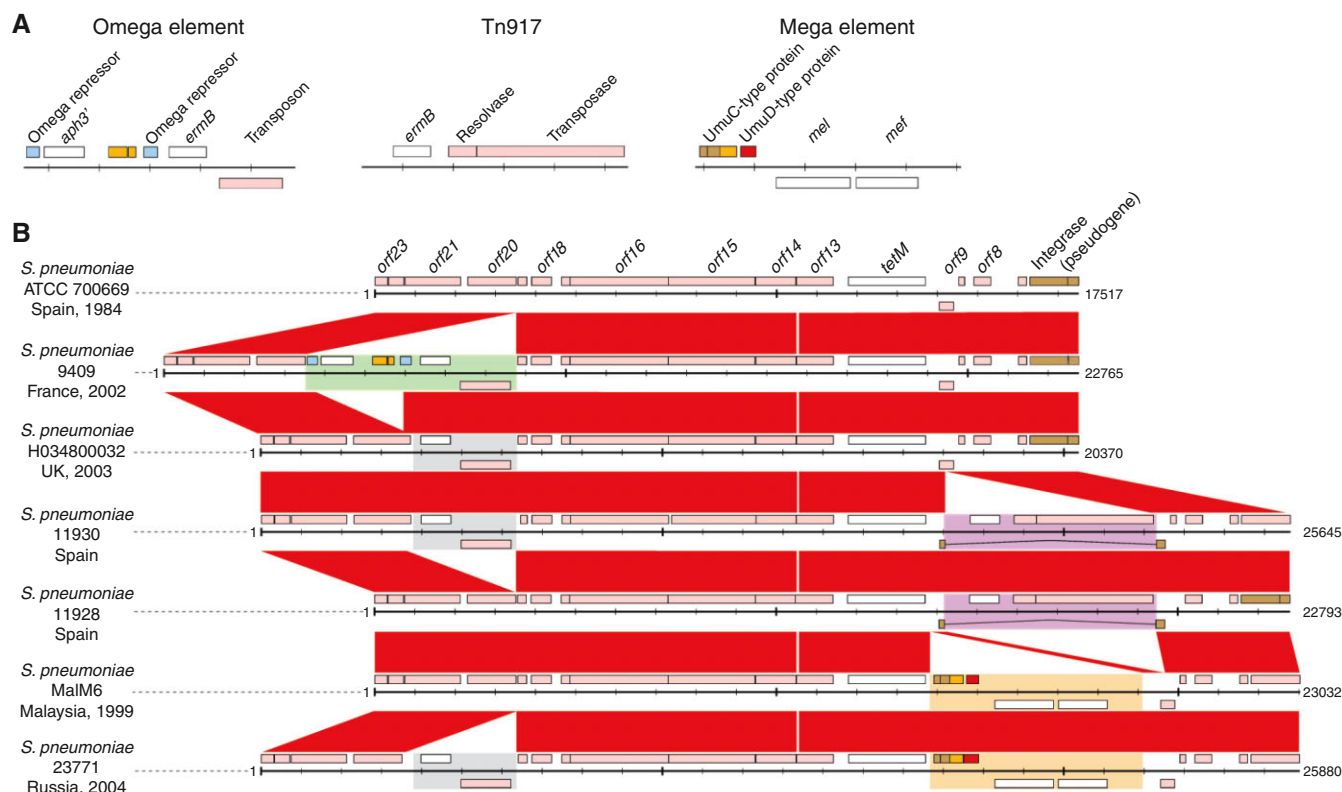


Fig. 3. Acquisition of macrolide-resistance cassettes. The three full-length resistance cassettes are shown in (A): the omega element, which carries an *aph3'* aminoglycoside-resistance gene and an *ermB* macrolide-resistance gene; Tn917, which carries just the *ermB* methylase; and the mega element, which carries the *mef/mel* macrolide efflux system. (B) A comparison of the different Tn916 variants in the PMEN1 lineage. Red bands between the sequences

indicate BLASTN matches. The omega element is shaded green when present at full length and shaded gray where present as a remnant resulting from a recombination between the omega repressor-encoding genes, which concomitantly leads to the fusion of an omega transcriptional repressor domain to the 3' of the *orf20* CDS. Tn917 is boxed in purple, with the two parts of *orf9*, into which it inserts, indicated on either side. The mega element is boxed in orange.

encoding genetic assembly) carries both an *ermB* gene and an aminoglycoside phosphotransferase, with the latter flanked by direct repeats of omega transcriptional repressor genes, and is found just downstream of *orf20*.

Rather than a single acquisition of these elements occurring, and the resulting clones spreading and replacing macrolide-sensitive isolates, all three elements appear to have been acquired multiple times across the phylogeny (fig. S10). The mega element is predominantly shared by isolates in clade I, although the *ermB*-encoding omega element appears to have been subsequently acquired on two occasions, and Tn917 has entirely superseded the mega element in one isolate. This is congruent with the known advantages of target methylation over drug efflux as a broader-spectrum resistance mechanism (28). In most instances of the omega element, only the *ermB*-encoding part remains; the aminoglycoside phosphotransferase appears to have been deleted through a recombination between the omega-encoding genes, which leaves only an omega domain-encoding open reading frame fused to *orf20* as a scar. This implies that the benefit of the aminoglycoside-resistance element may have not been sufficient to maintain it on the ICE.

Components of the accessory genome. Other than the insertion of these cassettes, the ICE itself is otherwise relatively unchanged throughout the population. In two cases, the 5' region of the element up to, and including, the lantibiotic synthesis machinery is deleted, whereas the self-immunity genes are retained (fig. S6). This deletion, which also removes the integrated chloramphenicol-resistance plasmid, is analogous to that observed in the pneumococcal pathogenicity island-1 of the PMEN1 lineage, in which all that remains are the immunity genes from a once-intact lantibiotic synthesis machinery (3). In two other cases, the ICE has been supplanted by alternative transposons, both of which are similar composites of Tn5252- and Tn916-type elements: In *S. pneumoniae* 11876, a wholesale replacement at the same locus entails the gain of an omega element at the expense of losing resistance to chloramphenicol (fig. S7), whereas, in isolate 11930, the new ICE inserts elsewhere in the chromosome and carries two *ermB* genes, as well as a chloramphenicol acetyltransferase (fig. S8). The only other identified conjugative element was an ICES1-type transposon shared by isolates 8140 and 8143 (fig. S9), and the only extra-chromosomal element present in the data set was the plasmid pSpn1 (29), found in isolate SA8.

The accessory genome is primarily composed of prophage sequence (fig. S11), with little evidence of much variation in the complement of metabolic genes. Viral sequences appear to be a transient feature of the pneumococcal chromosome (fig. S12), with few persisting long enough to be detected in related isolates. Four of the new prophage that could be assembled were found to insert into the competence pilus structural gene *comYC*, which lies within an operon shown to be essential for

competence in *S. pneumoniae* (30). In two cases where such phage appear to be shared through common descent by pairs of isolates, no recombination events can be detected that are unique to either member of the pair, consistent with a non-functional competence system in these isolates. Furthermore, assaying the competence of available lysogenic strains in vitro also suggested that these phage insertions abrogate the ability of their host to take up exogenous DNA (fig. S13).

Discussion. The ability to distinguish vertically acquired substitutions from horizontally acquired sequences is crucial to successfully reconstructing phylogenies for recombinogenic organisms such as *S. pneumoniae*. Phylogenies are in turn essential for detailed studies of events such as intercontinental transmission, capsule type switching, and antibiotic-resistance acquisition. Although current epidemiological typing methods have indicated that recombination is frequent among the pneumococcal population, they cannot sufficiently account for its impact on relations between strains at such high resolution. Only the availability of such a sample of whole-genome sequences makes it possible to adequately reconstruct the natural history of a lineage. The base substitutions used to construct the phylogeny have accumulated over about 40 years and occur, on average, once every 15 weeks. Recombinations happen at a rate about 1/10th as fast but introduce a mean of 72 SNPs each. The responses to the different anthropogenic selection pressures acting on this variation are distinct. The apparently weak selection by aminoglycosides and chloramphenicol has led to the occasional deletion of loci encoding resistance to these antibiotics. By contrast, resistance to macrolide antibiotics has been acquired frequently throughout the phylogeny, with selection strong enough to drive supplementation or replacement of the resistance afforded by the *mef* efflux pump with the broader-range resistance provided by *ermB*-mediated target modification. The response to vaccine selection is different and involves the depletion of the resident population before it can respond to the selection pressure and thereby opens the niche to isolates that already expressed nonvaccine serotypes. This is likely to reflect the high host population coverage of PCV7 in the USA, as opposed to macrolides or other antibiotics, and the relative likelihood of the recombination events that underlie these responses.

Over a few decades, this single pneumococcal lineage has acquired drug resistance and the ability to evade vaccine pressure multiple times, demonstrating the remarkable adaptability of recombinogenic bacteria such as the pneumococcus. PMEN1 is, nevertheless, only one lineage of this pathogen. Our relative ignorance of the forces that affect bacterial evolution over the long term is illustrated by BM4200 (31), a multidrug-resistant serotype 23F isolate of ST1010 sequenced as the outgroup for this analysis (Fig. 1). This isolate dates to 1978 but, despite its apparent similarity to PMEN1 strains, has been found very rarely

since then. Hence, this phenotype is not sufficient to guarantee success, an observation supported by the continued presence of successful, but susceptible, pneumococci in the population (32, 33). Improved understanding of the interplay between ecology and adaptation in other lineages through further focused sequencing programs may prove crucial to the future control of this, and other, diverse bacterial pathogens.

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34. We thank the participating surveillance networks, listed in table S1, and the core informatics, library-making, and sequencing teams at the Wellcome Trust Sanger Institute. Attending authors were grateful for the opportunity to discuss this project at the Permafrost conference. Sequence accession codes are given in tables S1 and S2. This work was funded by the Wellcome Trust.

Supporting Online Material

www.sciencemag.org/cgi/content/full/331/6016/430/DC1
Materials and Methods
Figs. S1 to S13
Tables S1 to S5
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The Genetic Landscape of the Childhood Cancer Medulloblastoma

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Medulloblastoma (MB) is the most common malignant brain tumor of children. To identify the genetic alterations in this tumor type, we searched for copy number alterations using high-density microarrays and sequenced all known protein-coding genes and microRNA genes using Sanger sequencing in a set of 22 MBs. We found that, on average, each tumor had 11 gene alterations, fewer by a factor of 5 to 10 than in the adult solid tumors that have been sequenced to date. In addition to alterations in the Hedgehog and Wnt pathways, our analysis led to the discovery of genes not previously known to be altered in MBs. Most notably, inactivating mutations of the histone-lysine N-methyltransferase genes *MLL2* or *MLL3* were identified in 16% of MB patients. These results demonstrate key differences between the genetic landscapes of adult and childhood cancers, highlight dysregulation of developmental pathways as an important mechanism underlying MBs, and identify a role for a specific type of histone methylation in human tumorigenesis.

Medulloblastomas (MBs) originate in the cerebellum, have a propensity to disseminate throughout the central nervous system, and are diagnosed in approximately 1 in 200,000 children less than 15 years old each year (1). Although aggressive multimodal therapy has improved the prognosis for children with MB, a substantial proportion of patients are currently incurable (2). Moreover, survivors often suffer considerable treatment-related morbidities, including neurocognitive deficits related to radiation therapy. New insights into the pathogenesis of these tumors are therefore sorely needed. Gene-based research has identified two subgroups of MBs, one associated with mutated genes within the Hedgehog pathway and the other associated with altered Wnt pathway genes (3, 4). Amplifications of *MYC* and the transcription factor *OTX2* (5–7), mutations in *TP53* (8), and a number of chromosomal alterations have also been identified in MBs. These discoveries have helped define the pathogenesis of MB and have improved our ability to identify patients who might benefit from therapies targeting these pathways. However, most MB patients do not have alterations in these genes, and the compendium of genetic alterations causing MB is unknown.

The determination of the human genome sequence and improvements in sequencing and bioinformatic technologies have recently permitted genome-wide analyses of human cancers. To date, the sequences of all protein-encoding genes have been reported in more than 80 human cancers (9–20), representing a variety of adult tumors. In this study, we provide a comprehensive sequence analysis of a solid tumor of childhood. Our data point to a major genetic difference between adult

and childhood solid tumors and provide new information to guide further research on this disease.

Sequencing strategy. In the first stage of our analysis, which we have called the “discovery screen,” 457,814 primers (table S1) were used to amplify and sequence 225,752 protein-coding exons, adjacent intronic splice donor and acceptor sites, and microRNA (miRNA) genes in 22 pediatric MB samples (17 samples extracted directly from primary tumors, 4 samples passaged in nude mice as xenografts, and 1 cell line) (tables S2 and S3). Seven metastatic MBs were selected for inclusion in the discovery screen to ensure that high-stage tumors were well represented in the study. One matched normal blood sample was sequenced as a control. These analyses corresponded to 50,191 transcripts representing at least 21,039 protein-coding genes present in the Ensembl, Consensus Coding Sequences (CCDS), and RefSeq databases and 715 miRNA genes from the miRBase database. A total of 404,438 primers were described in our previous publications, and an additional 53,376 primers were newly designed to amplify technically challenging genomic regions, miRNAs, or newly discovered Ensembl genes (table S1). The data were assembled for each amplified region and evaluated using stringent quality-control criteria, resulting in the successful amplification and sequencing of 96% of targeted amplicons and 95% of targeted bases in the 22 tumors. A total of 735 megabases (Mb) of tumor sequence data were generated in this manner.

After automated and manual curation of the sequence traces, regions containing potential sequence alterations (single base mutations and small insertions and deletions) not present in the reference genome or single-nucleotide polymorphism (SNP) databases were reamplified in both the tumor and

matched normal tissue DNA and analyzed either through sequencing by synthesis on an Illumina GAI instrument or by conventional Sanger sequencing (21). This process allowed us to confirm the presence of the mutation in the tumor sample and determine whether the alteration was somatic (i.e., tumor-specific). Additionally, mutations identified in the four xenograft samples were confirmed to be present in the corresponding primary tumors.

Analysis of sequence and copy number alterations. A total of 225 somatic mutations were identified in this manner (Table 1 and table S4). Of these, 199 (88%) were point mutations and the remainder were small insertions, duplications, or deletions, ranging from 1 to 48 base pairs in length. Of the point mutations, 148 (74%) were predicted to result in nonsynonymous changes, 42 (21%) were predicted to be synonymous, and 9 (5%) were located at canonical splice site residues that were likely to alter normal splicing. Of the 225 somatic mutations, 36 (16%) were predicted to prematurely truncate the encoded protein, either through newly generated nonsense mutations or through insertions, duplications, or deletions leading to a change in reading frame. The mutation spectrum observed for MB was similar to those seen in pancreatic,

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colorectal, glial, and other malignancies (22), with 5'-CG to 5'-TA transitions observed more commonly than other substitutions (Table 1). Such transitions are generally associated with endogenous processes, such as deamination of 5-methylcytosine residues, rather than exposure to exogenous carcinogens (23).

The distribution of somatic mutations among the 22 MBs is illustrated in Fig. 1. Two key differences were observed in this cancer as compared to the typical adult solid tumor. First, the average number of nonsilent somatic mutations (nonsynonymous missense, nonsense, indels, or splice site alterations) per MB patient was only 8.3, which is fewer by a factor of 5 to 10 than the average number of alterations detected in the previously studied solid tumor types (Table 1). Second, the proportion of nonsense mutations was more than twice as high as expected given the mutation spectra observed in this tumor type ($P < 1 \times 10^{-4}$, chi-squared test), and the relative fraction of nonsense, insertion, and duplication alterations was higher in MBs than in any of the adult solid tumors analyzed (Table 1) (21).

We evaluated copy number alterations using Illumina SNP arrays containing ~1 million probes in a set of 23 MBs, including all discovery screen samples. Using stringent criteria for focal amplifications and homozygous deletions, we identified 78 and 125 of these alterations, respectively, in these tumors (tables S5 and S6) (21). High-level amplifications indicate an activated oncogene within the affected region, whereas homozygous deletions may signal inactivation of a tumor suppressor gene. The total number of copy number changes affecting coding genes in each tumor is plotted in Fig. 1. Similar to the point mutation data, we found considerably fewer amplifications (an average of 0.4 per tumor) and homozygous deletions (an average of 0.8 per tumor) affecting coding genes than observed in adult solid tumors (which average 1.6 amplifications and 1.9 homozygous deletions) (18, 19, 24).

We next evaluated a subset of the mutated genes in an additional 66 primary MBs, including both pediatric and adult tumors (tables S2 and S3). This “prevalence screen” comprised sequence analysis of the coding exons of all genes that were either found to be mutated twice or more in the discovery screen or were mutated once in the discovery screen and had previously been reported to be mutated in other tumor types. Nonsilent somatic mutations were identified in 7 of these 15 genes (table S4). In the prevalence screen, the nonsilent mutation frequency was calculated to be 9.5 mutations per Mb, far higher than the rate found in the discovery screen (0.24 mutations per Mb; $P < 0.001$, Fisher’s exact test). The ratio of nonsilent to silent mutations in the prevalence screen was 24 to 1, which is more than 5 times as high as the 4.4 to 1 ratio determined in the discovery screen ($P < 0.01$, Fisher’s exact test). In addition, 23 of the 50 prevalence screen mutations (46%) were nonsense alterations or insertions or deletions that were expected to truncate the encoded protein. These data suggest that the genes selected for the prevalence screen were enriched for functionally important genes.

Frequent mutation of *MLL2* and *MLL3* in MB. Somatic mutations in tumor DNA can either provide a selective advantage to the tumor cell (driver mutations) or have no net effect on tumor growth (passenger mutations). A variety of methods are available to help distinguish whether a specific gene

or individual mutation is likely to be a driver. At the gene level, the passenger probability score corresponds to a metric reflecting the frequency of mutations, including point mutations, indels, amplifications, and homozygous deletions, normalized for sequence context as well nucleotide composi-

Table 1. Summary of somatic sequence mutations in five tumor types.

	Medulloblastoma*	Pancreas†	Glioblastoma‡	Colorectal§	Breast§
Number of samples analyzed	22	24	21	11	11
Number of mutated genes	218	1007	685	769	1026
Number of nonsilent mutations	183	1163	748	849	1112
Missense	130 (71.0)	974 (83.7)	622 (83.2)	722 (85)	909 (81.7)
Nonsense	18 (9.8)	60 (5.2)	43 (5.7)	48 (5.7)	64 (5.8)
Insertion	5 (2.7)	4 (0.3)	3 (0.4)	4 (0.5)	5 (0.4)
Deletion	14 (7.7)	43 (3.7)	46 (6.1)	27 (3.2)	78 (7.0)
Duplication	7 (3.8)	31 (2.7)	7 (0.9)	18 (2.1)	3 (0.3)
Splice site or untranslated region	9 (4.9)	51 (4.4)	27 (3.6)	30 (3.5)	53 (4.8)
Average number of nonsilent mutations per sample	8	48	36	77	101
Observed/expected number of nonsense alterations¶	2.48	1.18	1.00	1.25	1.37
Total number of substitutions#	199	1486	937	893	1157
Substitutions at C:G base pairs					
C:G to T:A**	109 (54.8)	798 (53.8)	601 (64.1)	534 (59.8)	422 (36.5)
C:G to G:C**	12 (6.0)	142 (9.6)	67 (7.2)	61 (6.8)	325 (28.1)
C:G to A:T**	41 (20.6)	246 (16.6)	114 (12.1)	130 (14.6)	175 (15.1)
Substitutions at T:A base pairs					
T:A to C:G**	19 (9.5)	142 (9.6)	87 (9.3)	69 (7.7)	102 (8.8)
T:A to G:C**	14 (7.0)	79 (5.3)	24 (2.6)	59 (6.6)	57 (4.9)
T:A to A:T**	4 (2.0)	77 (5.2)	44 (4.7)	40 (4.5)	76 (6.6)
Substitutions at specific dinucleotides					
5'-CpG-3'**	85 (42.7)	563 (37.9)	404 (43.1)	427 (47.8)	195 (16.9)
5'-TpC-3'**	14 (7.0)	218 (14.7)	102 (10.9)	99 (11.1)	395 (34.1)

*Based on 22 tumors analyzed in the current study. †Based on 24 tumors analyzed in (18). ‡Based on 21 nonhypermutable tumors analyzed in (19). §Based on 11 breast and 11 colorectal tumors analyzed in (16, 17). ||Numbers in parentheses refer to percentage of total nonsilent mutations. ¶Ratio of observed to expected nonsense alterations is dependent on mutation spectra in each tumor type (21). #Includes synonymous as well as nonsynonymous point mutations identified in the indicated study. **Numbers in parentheses refer to percentage of total substitutions.

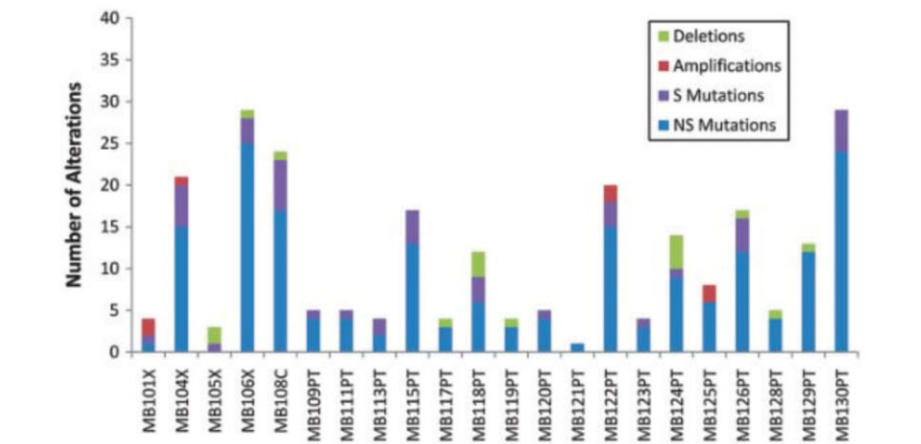


Fig. 1. Number of genetic alterations detected through sequencing and copy number analyses in each of the 22 cancers. NS, nonsilent mutations (including nonsynonymous alterations, insertions, duplications, deletions, and splice site changes); S, silent mutations; deletions, gene-containing regions absent in tumor samples; amplifications, gene-containing regions focally amplified at levels > 10 copies per nucleus (21).

tion and length of the gene. The lower the passenger probability score, the less likely it is that mutations in the specific gene represent passengers. Passenger probability scores of the candidate cancer genes (*CAN*-genes) identified in MB are listed in Table 2.

At the individual mutation level, the Cancer-Specific High-Throughput Annotation of Somatic Mutations (CHASM) score is a metric reflecting the likelihood that a missense mutation alters the normal function of the respective protein and provides a selective advantage to the tumor cell (25). The CHASM score is based on 73 biochemical features, including conservation of the wild-type amino acid and the mutation's predicted effects on secondary structure. The CHASM score for each mutation observed in this study and the associated *P* value are listed in table S4. Nonsense mutations, as well as small insertions or deletions that disrupt the reading frame, are likely to disrupt function and are assigned a score of 0.001 in this table. About 36% of the evaluated mutations in MB were predicted to disrupt gene function using this approach, a proportion higher than observed in the adult tumor types analyzed to date (21).

Finally, we evaluated the discovery screen mutational data (including both sequence and copy number alterations) at a higher "gene set" level. There is now abundant evidence that alterations of driver genes can be productively organized according to the biochemical pathways and biological processes through which they act. The number of gene sets that define these pathways and

processes is much less than the number of genes and can provide clarity to lists of genes identified through mutational analyses. In the current study, we used a recently described approach that scores each gene set at the patient rather than the gene level and is more powerful than conventional gene-oriented approaches (21, 26). The most statistically significant pathways and biologic processes highlighted by this gene-set analysis are depicted in table S7. Of these, two—the Hedgehog and Wnt signaling pathways—have been previously shown to play a critical role in MB development. In the Hedgehog pathway, *PTCH1* was mutated in 15 of 88 (17%) tumors, and in the Wnt pathway, *CTNNB1* was mutated in 11 of 88 (13%) tumors (table S4).

Notably, however, the pathways most highly enriched for genetic alterations had not previously been implicated in MB. These involved genes responsible for chromatin remodeling and transcriptional regulation, particularly the histone-lysine N-methyltransferase *MLL2*. Eighteen of the 88 (20%) tumors harbored a mutation in a gene within these pathways or in a related gene member: the histone-lysine N-methyltransferases *MLL2* (mutated in 12 tumors) and *MLL3* (3 tumors); the SWI/SNF-related matrix-associated actin-dependent regulator of chromatin members *SMARCA4* (3 tumors) and *ARID1A* (1 tumor); and the histone lysine demethylase *KDM6B* (1 tumor). The mutations in these genes could be clearly distinguished from passenger alterations. In *MLL2*, for example, 8 of the 12 mutations (67%) were predicted to truncate the encoded

proteins as a result of nonsense mutations, out-of-frame indels, or splice site mutations. In contrast, only 32 of the 222 mutations (14%) not affecting core genes of the Hedgehog, Wnt, or *MLL2*-related pathways (*PTCH1*, *CTNNB1*, *MLL2*, *MLL3*, *SMARCA4*, *ARID1A*, and *KDM6B*) resulted in predicted protein truncations ($P < 0.001$, Fisher's exact test). The probability that by chance alone 11 of the 15 mutations in the two histone methyltransferase genes would cause truncations is very small ($P < 0.001$, binomial test). All truncating mutations in *MLL2* and *MLL3* were predicted to result in protein products lacking the key methyltransferase domain (Fig. 2). These data not only provide strong evidence that these pathways are important to MBs, but they also show that *MLL2* and *MLL3* are, on the basis of genetic criteria, tumor suppressor genes that are inactivated by mutation.

Discussion. These data provide a comprehensive view of a solid tumor arising in children. The most impressive difference between this tumor type and those affecting adults is the number of genetic alterations observed. This result could not have been predicted on the basis of previous evidence (27). In fact, at the karyotypic level, the incidence of chromosomal changes in MBs is often described as high as that in adult solid tumors [reviewed in (27)].

What does the smaller number of mutations reveal about the tumorigenesis of MBs? Most mutations observed in adult tumors are predicted to be passenger alterations (19). Passenger mutations provide an evolutionary clock that precisely records the number of divisions that a cell has undergone during both normal development and tumor progression. Therefore, the cell division number is linearly related to the number of passenger mutations detected in a tumor (28). This concept is consistent with the positive correlation we identified between increasing patient age and the number of mutations found in their MBs. This relationship was observed for both the mutations detected in the exomes of the discovery screen tumors ($r = 0.73$, $P < 0.01$) and the number of alterations observed in the subset of 15 genes analyzed in the discovery and prevalence screen samples ($r = 0.32$, $P < 0.01$) (tables S8 and S9). Even if we assume that all but one of the mutations in each MB is a passenger, the number of passenger mutations in MBs is still substantially smaller than the number of passenger mutations in adult solid tumors (16–19), implying that a smaller number of cell divisions is required to reach clinically detectable tumor size in MBs. These data therefore suggest that fewer driver mutations are required for MB tumorigenesis and that driver mutations in MB confer a greater selective advantage than those of adult solid tumors.

Previously, most insights into the molecular basis of MB emerged from the study of hereditary tumor syndromes (27), including Gorlin syndrome, caused by germline mutations of *PTCH1*; Turcot syndrome, caused by germline mutations of *APC*; and Li-Fraumeni syndrome, caused by germline mutations of *TP53*. In our study, we found both *PTCH1* and *TP53* to be somatically mutated in MBs (Table 2 and table S4) at frequencies similar

Table 2. Medulloblastoma *CAN*-genes.*

Gene	Number of mutations	Number of amplifications	Number of deletions	Passenger probability
<i>PTCH1</i>	22 / 88	0 / 23	0 / 23	<0.001
<i>MLL2</i>	12 / 88	0 / 23	0 / 23	<0.001
<i>CTNNB1</i>	11 / 88	0 / 23	0 / 23	<0.001
<i>TP53</i>	6 / 88	0 / 23	0 / 23	<0.001
<i>MYC</i>	0 / 88	3 / 23	0 / 23	<0.001
<i>PTEN</i>	3 / 88	0 / 23	0 / 23	0.008
<i>OTX2</i>	0 / 88	2 / 23	0 / 23	0.015
<i>SMARCA4</i>	3 / 88	0 / 23	0 / 23	0.104
<i>MLL3</i>	3 / 88	0 / 23	0 / 23	0.104

**CAN*-genes were defined as those having at least two nonsilent alterations in the samples analyzed. Passenger probabilities were calculated as described in (21). The denominators refer to the number of tumors evaluated: 88 tumors were sequenced for mutations, and 23 tumors were analyzed for copy number alterations.

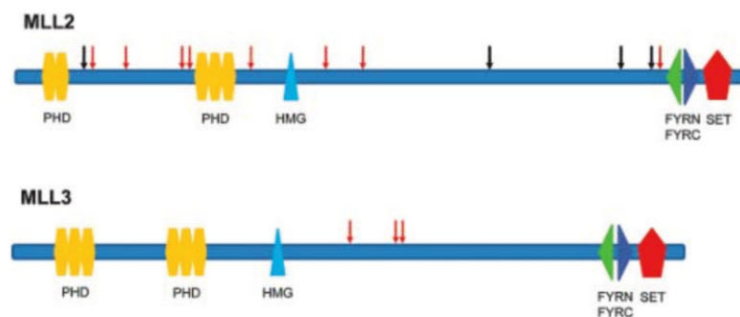


Fig. 2. Somatic mutations in *MLL2* and *MLL3* genes. Nonsense mutations and out-of-frame insertions and deletions are indicated as red arrows; missense mutations are indicated as black arrows. PHD, plant homeodomain finger; HMG, high mobility group box; FYRN, FY-rich N-terminal domain; FYRC, FY-rich C-terminal domain; SET, Su(var)3-9 Enhancer-of-zeste Trithorax methyltransferase domain.

to those observed in earlier studies. We also identified amplifications of *MYC* and *OTX2*, both previously implicated in MB (5–7).

The ability to investigate the sequence of all coding genes in MBs has also revealed mutated genes not previously implicated in MBs (table S4). Among these, *MLL2* and *MLL3* were of greatest interest, because the frequency of inactivating mutations unequivocally establishes them as MB tumor suppressor genes. This genetic evidence is consistent with functional studies showing that knock-out of murine *MLL3* results in ureteral epithelial cancers (29). These genes are large and have been reported in the Catalogue of Somatic Mutations in Cancer (COSMIC) database to be altered in occasional cancers, but not at a sufficiently high frequency to distinguish them from passenger alterations (and with no evidence of a high fraction of inactivating mutations) (30). Interestingly, inactivating germline mutations of *MLL2* have recently been identified as a cause of Kabuki syndrome, a multiple malformation disorder without known cancer predisposition (31).

The general role of genes controlling histone methylation has become increasingly recognized as a common feature of human cancers. For example, inactivating mutations of the histone H3K27 demethylase gene *UTX* have been observed in multiple myelomas, esophageal cancers, and renal cell cancers (32). In addition, a small fraction of renal cell cancers contain mutations in the histone methyltransferase gene *SETD2* and the histone demethylase gene *JARID1C* (33), and the histone methyltransferase gene *EZH2* has been found to be mutated in non-Hodgkin's lymphomas (34). Most recently, frequent mutations of the chromatin remodeling gene *ARID1A* have been discovered in ovarian clear cell carcinomas (20, 35); of note, one *ARID1A* mutation was discovered in our MB patients (table S4). A link between histone methylation genes (although not *MLL2* or *MLL3*) and MB has also previously been hypothesized based on the

observation that copy number alterations affecting chromosomal regions containing histone methyltransferases or demethylases occur in a subset of MBs (36).

The mechanism(s) through which *MLL* genes contribute to tumorigenesis are not known, but some clues can be gleaned from the literature. The *MLL* family of histone H3K4 trimethylases includes seven genes (*MLL1*, *MLL2*, *MLL3*, *MLL4*, *MLL5*, *SET1A*, and *SET1B*) (37). *MLL* family genes have been shown to regulate *HOX* gene expression (38, 39), and an attractive possibility is that they normally down-regulate *OTX2*, an MB oncogene (6, 7, 40). Another possibility is suggested by the observation that β -catenin brings *MLL* complexes to the enhancers of genes regulating the Wnt pathway, thereby activating their expression (41). A third possibility is that *MLL* family genes are important for transcriptional regulation of normal brain development and differentiation (42) and that their disruption may lead to aberrant proliferation of precursor cells.

The identification of *MLL2* and *MLL3* as frequently inactivated MB genes supports the concept that MB is fundamentally characterized by dysregulation of core developmental pathways (43). Although alterations of classic cancer genes (e.g., *TP53*, *MYC*, and *PTEN*) were also identified in these childhood tumors, our sequence analysis demonstrated that mutations of genes involved in normal developmental processes, such as *MLL* family genes and Hedgehog and Wnt pathway genes, were much more frequent. The fact that a relatively small number of somatic mutations is sufficient for MB pathogenesis as compared to adult solid tumors provides further evidence that the temporally restricted subversion of normal cerebellar development is critical in the development of these tumors. This is consistent with the observation that the incidence of MB decreases markedly after childhood, with the tumors becoming quite rare after the age of 40 years (1). It

will be interesting to determine whether genetic alterations in developmental pathways are a key feature of all childhood malignancies.

The development of an improved classification system for MB that could be used to guide targeted risk-adapted therapy to patients is a primary goal of current MB research. The designation of specific histologic subtypes of MB has proven to be of some prognostic value. For example, large-cell/anaplastic MBs, which are aggressive tumors often associated with *MYC* amplification, carry a relatively poor prognosis (44), whereas desmoplastic MBs, which frequently have alterations of *PTCH1* or other Hedgehog pathway genes (4), are more easily treatable. However, molecular studies have revealed that these histologic subtypes are biologically heterogeneous (3); in addition, most MBs are of the classic subtype and do not have defining molecular alterations. Our results add an additional layer of complexity to these classifications. Although activation of the Wnt and Hedgehog pathways are generally considered to define two MB subtypes (3), our data revealed that these groups overlap, because two adult MBs were found to contain mutations of both *PTCH1* and *CTNNB1* (tables S2 and S4). Similarly, *MLL2/MLL3* mutations do not appear exclusive to any known subset of MBs: Mutations were identified in both pediatric and adult MBs and were found in all histologic subtypes (although they were most common in large-cell/anaplastic MBs) (Table 3 and tables S9 and S10). In addition, the frequency of *MLL2/MLL3* mutations was observed to be similar in *PTCH1*- or *CTNNB1*-mutated MBs (4/24, 17%) as compared to MBs without mutations in *PTCH1* or *CTNNB1* (10/64, 16%). Further studies of these genes in larger number of MBs that have been analyzed for pathologic subtypes will be needed to clarify the molecular classification of this tumor.

We conclude that each MB is driven by a small number of driver mutations, and in our cohort, the

Table 3. Characteristics of medulloblastomas with mutations in *MLL2*-related genes.*

Tumor ID	<i>MLL2</i> mutation	<i>MLL3</i> mutation	<i>SMARCA4</i> mutation	<i>ARID1A</i> mutation	<i>KDM6B</i> mutation	Patient age (years)	MB subtype	<i>PTCH1</i> mutation	<i>CTNNB1</i> mutation
MB104X	Nonsense	Nonsense				8	Classic		
MB108C			Missense			Unknown	Unknown		
MB115PT	Missense					5	Classic		
MB118PT				Frameshift		9	Classic		Yes
MB124PT	Frameshift					9	Large cell/anaplastic		
MB126PT	Missense					11	Large cell/anaplastic		
MB127PT	Frameshift					10	Unknown		
MB129PT					Nonsense	Unknown	Unknown		
MB130PT	Frameshift					Unknown	Unknown	Yes	
MB135PT	Frameshift					Unknown	Unknown		
MB205PT		Nonsense				11	Classic		
MB216PT	Missense					33	Large cell/anaplastic		
MB231PT			Missense			18	Classic		Yes
MB245PT	Frameshift					9	Classic		
MB246PT	Missense		Missense			7	Classic		Yes
MB249PT		Nonsense				10	Classic		Yes
MB251PT	Frameshift					26	Large cell/anaplastic	Yes	
MB253PT	Nonsense					32	Nodular/desmoplastic		

*All genes reported in the table were determined to be wild type unless otherwise indicated.

gene set most highly enriched for alterations included *MLL2*. However, there are several limitations to our study. Although in a few cases we have identified two or three bona fide cancer genes that are mutated in individual MBs, other cases show no mutations of any known cancer gene and only one alteration of any gene (Fig. 1 and table S4). Several explanations for the relative absence of genetic alterations in occasional MBs can be offered. First, despite the use of classic Sanger sequencing, a small fraction of the exome cannot be examined, either because of a very high GC content or of homology to highly related genes. Second, it is possible that mutations in the noncoding regions of the genome could occur, and these would not be detected. Third, copy-neutral genetic translocations, not evaluated in our study, could be present in those tumors with very few point mutations, amplifications, or homozygous deletions. Fourth, it is possible that low copy number gains or loss-of-heterozygosity (LOH) of specific regions containing histone-modifying genes could mimic the intragenic mutations that we observed (36). Finally, it is possible that heritable epigenetic alterations are responsible for initiating some MBs. The last explanation, involving covalent changes in chromatin proteins and DNA, is intriguing given the new data on *MLL2* in this tumor type. It should thus be informative to characterize the methylation status of histones and DNA in MBs with and without *MLL2/MLL3* gene alterations, as well as to determine the expression changes resulting from these gene mutations. These data highlight the important connection between genetic alterations in the cancer genome and epigenetic pathways and provide potentially new avenues for research and disease management in MB patients.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1198056/DC1
Materials and Methods
Tables S1 to S10
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REPORTS

Rotational Symmetry Breaking in the Hidden-Order Phase of URu₂Si₂

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A second-order phase transition is characterized by spontaneous symmetry breaking. The nature of the broken symmetry in the so-called "hidden-order" phase transition in the heavy-fermion compound URu₂Si₂, at transition temperature $T_h = 17.5$ K, has posed a long-standing mystery. We report the emergence of an in-plane anisotropy of the magnetic susceptibility below T_h , which breaks the four-fold rotational symmetry of the tetragonal URu₂Si₂. Two-fold oscillations in the magnetic torque under in-plane field rotation were sensitively detected in small pure crystals. Our findings suggest that the hidden-order phase is an electronic "nematic" phase, a translationally invariant metallic phase with spontaneous breaking of rotational symmetry.

A second-order phase transition generally causes a change in symmetry, such as rotational, gauge, or time-reversal symmetry.

An order parameter can then be introduced to describe the low-temperature ordered phase with a reduced symmetry. The heavy-fermion com-

pound URu₂Si₂ undergoes a second-order phase transition at $T_h = 17.5$ K, which is accompanied by large anomalies in thermodynamic and transport properties (1–3). Because the nature of the associated order parameter has not been elucidated, the low-temperature phase is referred to as the hidden-order phase. It is characterized by several remarkable features. No structural phase transition is observed at T_h . A tiny magnetic moment appears ($M_0 \approx 0.03\mu_B$, where μ_B is the Bohr magneton) below T_h (4), but it is far too

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small to explain the large entropy released during the transition and seems to have an extrinsic origin (5–7). An electronic excitation gap is formed on a large portion of the Fermi surface and most of the carriers (~90%) disappear (8–10); a gap is also formed in the incommensurate magnetic excitation spectrum (11).

The nature of the hidden order cannot be determined without understanding which symmetry is being broken. Several microscopic models, including multipole ordering (12–16), spin-density wave formation (17–19), orbital currents (20), and helicity order (21), have been proposed. However, despite intensive experimental and theoretical studies, this remains an open question.

The measurement of the magnetic torque $\tau = \mu_0 \mathbf{M} \mathbf{V} \times \mathbf{H}$ has a high sensitivity for detecting magnetic anisotropy. Here V is the sample volume, $\mathbf{M}$ is the induced magnetization, $\mathbf{H}$ is the magnetic field, and μ_0 is the permeability of vacuum. In particular, torque measurements performed for a range of directions of $\mathbf{H}$ spanning the tetragonal ab plane in URu_2Si_2 provide a stringent test of whether the hidden-order parameter breaks the crystal four-fold symmetry. In such a geometry, τ is a periodic function of double the azimuthal angle ϕ measured from the a axis:

$$\tau_{2\phi} = \frac{1}{2} \mu_0 H^2 V [\chi_{aa} - \chi_{bb}] \sin 2\phi - 2\chi_{ab} \cos 2\phi \quad (1)$$

where the susceptibility tensor χ_{ij} is given by $M_i = \sum_j \chi_{ij} H_j$ (Fig. 1). In a system with tetragonal symmetry, $\tau_{2\phi}$ should be zero because $\chi_{aa} = \chi_{bb}$ and $\chi_{ab} = 0$. Finite values of $\tau_{2\phi}$ may appear if a new electronic or magnetic state emerges that breaks the tetragonal symmetry. In such a case, rotational symmetry breaking is revealed by $\chi_{aa} \neq \chi_{bb}$ or $\chi_{ab} \neq 0$, depending on the orthorhombicity direction.

Figure 2A depicts the torque measured in field $\mathbf{H}$, the orientation of which is varied within a plane that includes the c axis (Fig. 2A, inset). Both below and above T_h , the curves are perfectly sinusoidal and can be fitted with $\tau(T, H, \theta) = A_{2\theta}(T, H) \sin 2\theta$, where A is the amplitude, θ is the polar angle, and T is absolute temperature. In our carefully selected crystals (22), no hysteresis is observed, indicating no detectable ferromagnetic impurities (the hysteresis component is less than 0.01% of the total torque). In this geometry, the difference $\Delta\chi_{ca}$ between the c axis and in-plane susceptibilities yields a two-fold oscillation term $\tau_{2\theta}(\theta, T, H)$ with respect to θ rotation,

$$\tau_{2\theta} = \frac{1}{2} \mu_0 H^2 V \Delta\chi_{ca} \sin 2\theta \quad (2)$$

The H -linear dependence of $A_{2\theta}(H, T)/H$ with a negligible y intercept (Fig. 2B) indicates a field-independent magnetic susceptibility—that is, a purely paramagnetic response. This also

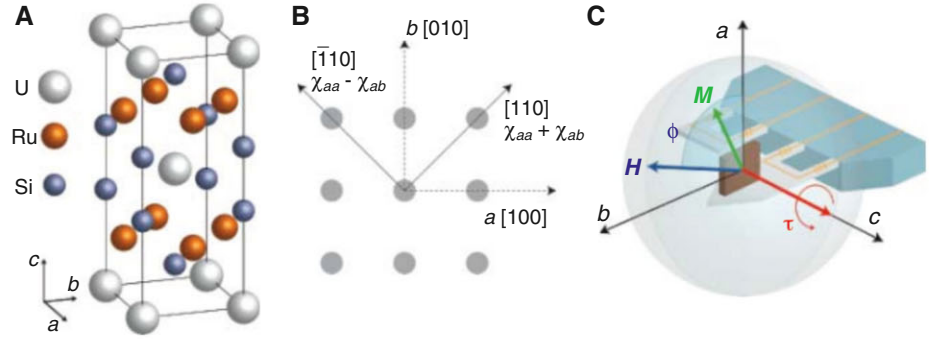


Fig. 1. (A) The tetragonal crystal structure of URu_2Si_2 . (B) U atom arrangement in the ab plane. The relevant axes and the susceptibility components for the $\chi_{aa} = \chi_{bb}$ case are also shown. (C) Schematics of in-plane ϕ -scan measurements. The magnetic field $\mathbf{H}$ was applied in the ab plane with high alignment precision (within 0.02°).

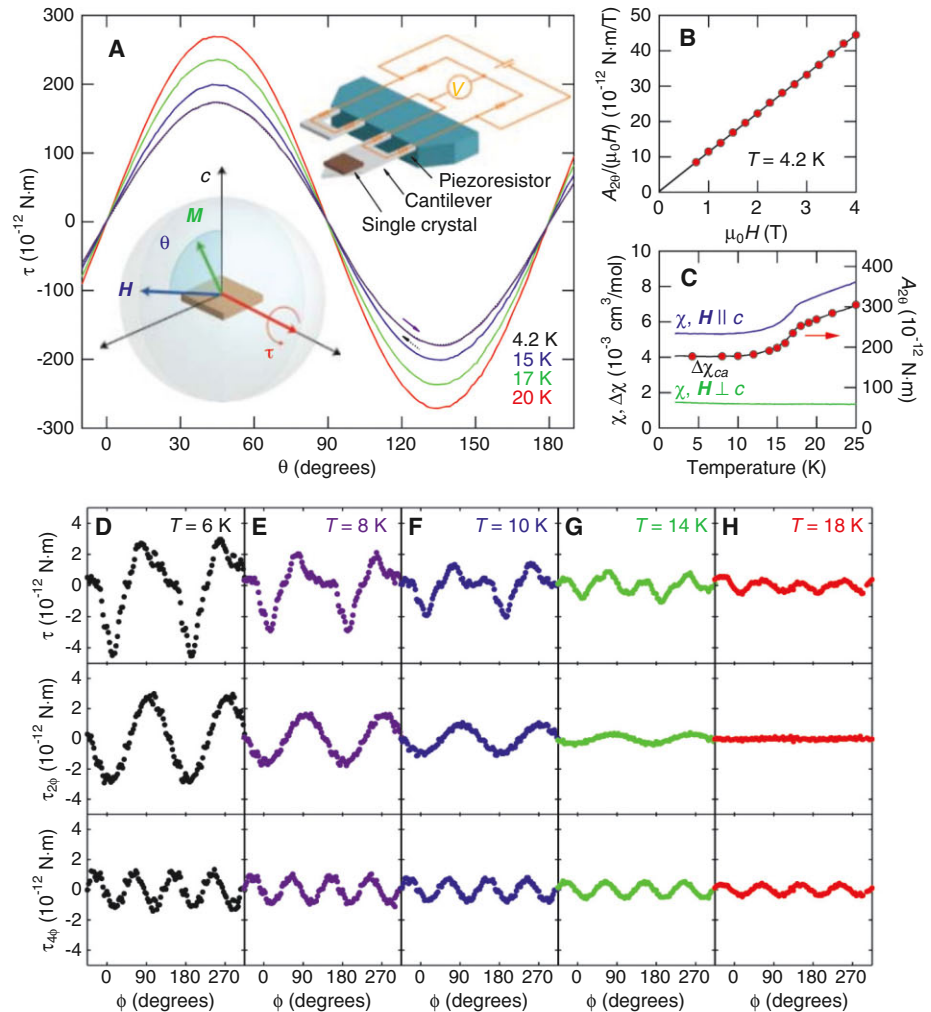


Fig. 2. Magnetic torque curves measured at $|\mu_0 \mathbf{H}| = 4$ T. (A) Torque $\tau(\theta)$ as a function of the polar angle θ (lower inset) measured at several temperatures. Torque curves measured by rotating $\mathbf{H}$ in clockwise (dotted line) and anticlockwise (solid line) directions coincide. Upper inset illustrates the experimental configuration for $\tau(\theta)$ measurements. (B) Magnetic field dependence of the amplitude of the two-fold oscillation of the torque divided by the field, $A_{2\theta}/(\mu_0 H)$, at $T = 4.2$ K. (C) Left axis: Temperature dependence of the susceptibility χ for $\mathbf{H} \perp c$ (green) and $\mathbf{H} \parallel c$ (blue). Their difference $\Delta\chi_{ca}$ is shown in black. Right axis: Temperature dependence of $A_{2\theta}$ (red circles). (D to H) Upper panels show raw torque curves $\tau(\phi)$ as a function of the azimuthal angle ϕ at several temperatures. Middle and lower panels show the two-fold $\cos 2\phi$ and four-fold $\sin 4\phi$ components of the torque curves, respectively, obtained from Fourier analysis of the raw torque curves.

reinforces the absence of ferromagnetic impurities. To check the consistency of the data, we also measured the susceptibility of a different single crystal of URu₂Si₂ in the same batch by means of a superconducting quantum interference device (SQUID) magnetometer (Fig. 2C). The amplitudes of $\Delta\chi_{ca}$ obtained in the two types of measurements coincide in both the hidden-order and paramagnetic states.

Having established the evidence of the purely paramagnetic response in our single crystals, we now consider the in-plane torque measured in **H** rotating within the *ab* plane. To exclude two-fold oscillations appearing as a result of misalignment, **H** is precisely applied in the *ab* plane within an error of less than 0.02° by controlling two superconducting magnets and a rotating stage (22) (Fig. 1C). Shown in Fig. 2, D to H, is the temperature evolution of the torque $\tau(\phi)$ at 4 T in the hidden-order phase. All torque curves are perfectly reversible with respect to the field rotation direction; $\tau(\phi)$ can be decomposed as $\tau = \tau_{2\phi} + \tau_{4\phi} + \tau_{6\phi} + \dots$, where $\tau_{2n\phi} = A_{2n\phi} \sin 2n(\phi - \phi_0)$ is a term with $2n$ -fold symmetry with $n = 1, 2, \dots$. In the middle and lower panels of Fig. 2, D to H, the two-fold and four-fold components obtained from the Fourier analysis are displayed.

Our results show that the two-fold oscillation is distinctly observed in the hidden-order state, whereas it is absent in the paramagnetic phase at $T = 18$ K slightly above T_h (middle panels of Fig. 2, D to H). Indeed, the torque curves shown in the upper panels become asymmetric with respect to 90° rotations below T_h . We note that the observed four-fold oscillations $\tau_{4\phi}$ (and higher-order terms) arise primarily from the nonlinear susceptibilities (23). The presence of the two-fold oscillation, which follows the functional form $\tau_{2\phi} = A_{2\phi} \cos 2\phi$, clearly demonstrates that $\chi_{ab} \neq 0$, whereas $\chi_{aa} = \chi_{bb}$. Figure 3A depicts the temperature dependence of $|A_{2\phi}|/V$, which indicates sizable in-plane anisotropy

$2\chi_{ab}/\chi_{aa}$ at low temperatures. The two-fold amplitude becomes nonzero precisely at $T_h = 17.5$ K (Fig. 3A, inset). This result, together with the absence of hysteresis in the torque curves, rules out the possibility that very tiny ferromagnetic impurities are the origin of the two-fold symmetry (22). Moreover, $|A_{2\phi}|$ grows rapidly with decreasing temperature in a manner quite different from $\Delta\chi_{ca}(T)$ (Fig. 2C). This indicates that a misalignment of **H** from the *ab* plane is unlikely to be the origin of the two-fold symmetry. On the basis of this reasoning, we conclude that the amplitude of two-fold oscillations is a manifestation of intrinsic in-plane anisotropy of the susceptibility (Fig. 1B):

$$\chi[110] = \chi_{aa} + \chi_{ab} \neq \chi[\bar{1}10] = \chi_{aa} - \chi_{ab} \quad (3)$$

The in-plane anisotropy that sets in precisely at T_h indicates that the rotational symmetry is broken in the hidden-order phase. The temperature dependence of $|A_{2\phi}| \propto \chi_{ab}$ near T_h is fitted with $\chi_{ab} \propto c(T_h - T) + c'(T_h - T)^2$ much better than with a purely quadratic fit (Fig. 3A, inset). The leading T -linear term near T_h (Fig. 3B) is naturally expected from the standard Landau theory of second-order transition when χ_{ab} is described by the squared term of an order parameter $\eta \propto (T_h - T)^{1/2}$. Thus, the observed in-plane anisotropy $2\chi_{ab}(T)/\chi_{aa}$ implies that the hidden-order parameter η breaks the four-fold tetragonal symmetry.

Why has such an in-plane magnetic anisotropy not been reported before? To address this, we measured several samples with different sizes. In millimeter-sized crystals, we observed no difference between $\chi[110](T)$ and $\chi[\bar{1}10](T)$, but in samples with a smaller volume V , a nonzero $2\chi_{ab} \propto |A_{2\phi}|/V$ appeared (Fig. 3A). This may imply that the hidden-order phase forms domains with different preferred directions in the *ab* plane, which may be a natural consequence

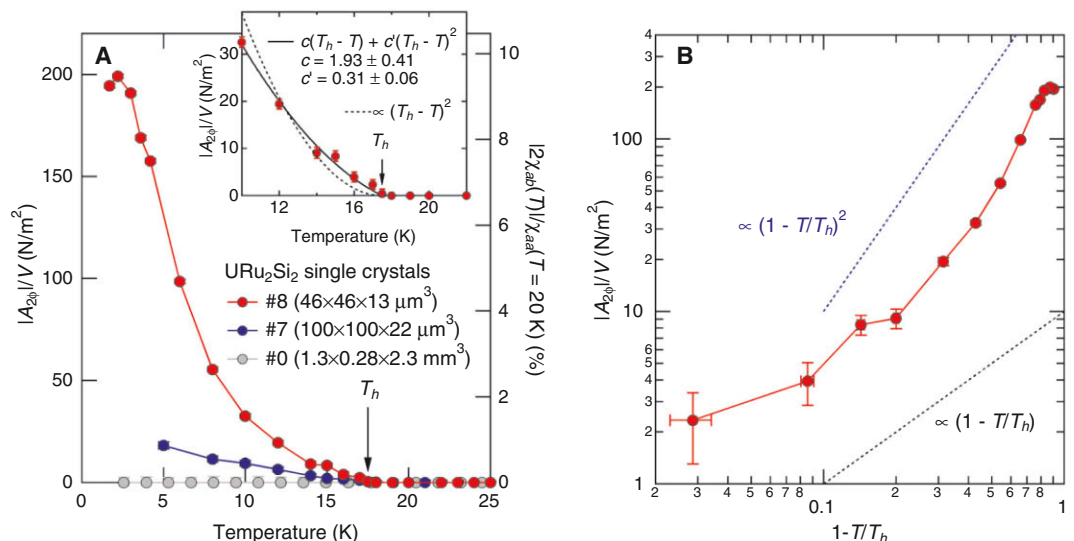
of the tetragonal crystal structure (with possible small undetected orthorhombicity in the domain). A domain size on the order of tens of micrometers would explain both our results and the difficulties in observing this effect. The fact that the torque curves remain unchanged for field-cooling conditions at different field angles (fig. S2) implies that the formation of such domains is predominantly determined and strongly pinned by the underlying crystal conditions, such as internal stress or disorder.

We now comment on the relevance of the present observation to other experimental results. The nuclear magnetic resonance (NMR) spectra have been reported to exhibit an anomalous broadening in the hidden-order phase for **H**//*ab* (5, 6, 24). This phenomenon has been discussed in terms of the orbital currents associated with the breaking of time-reversal symmetry (20). The present results provide an alternative explanation to this: Finite values of χ_{ab} give rise to the broadening of the NMR spectrum, which is estimated to be ~1 Oe at 4 T (22), roughly the same as the reported values (6). We also note that some of the nuclear quadrupole frequency ν_Q measurements (25), which are sensitive to the local charge density, report a small change in the slope of $\nu_Q(T)$ at the Ru site below T_h . As for the crystal structure, no discernible lattice distortions have been reported. This may be a result of the weak coupling between the lattice and electronic excitations relevant to the two-fold symmetry. The symmetry of superconductivity, which appears at $T_c = 1.4$ K and is embedded in the hidden-order phase, should also be restricted by the found rotational asymmetry. Recently, a chiral *d*-wave superconducting state of the form

$$\sin \frac{k_z}{2} c \left(\sin \frac{k_x + k_y}{2} a + i \sin \frac{k_x - k_y}{2} a \right) \quad (4)$$

has been proposed (10). The rotational symmetry breaking implies that the states including

Fig. 3. (A) Temperature dependence of two-fold oscillation amplitude divided by the sample volume $|A_{2\phi}|/V$ measured for sample 7 (blue circles) and sample 8 (red circles). The normalized in-plane susceptibility anisotropy $2\chi_{ab}/\chi_{aa} = (\chi[110] - \chi[\bar{1}10])/\chi[100]$ is evaluated in the right axis. SQUID data for the large crystal, sample 0, are also shown (gray circles) in the same scale. Inset: the data for sample 8 near $T_h = 17.5$ K are fitted to $c(T_h - T) + c'(T_h - T)^2$ (solid line) and the quadratic dependence $\propto (T_h - T)^2$ (dashed line). **(B)** Temperature variation of $|A_{2\phi}|/V$ for sample 8 on a log-log scale. The black and blue dotted lines represent linear and quadratic temperature dependence, respectively.



$\sin [(k_x + k_y)/2]a$ and $\sin [(k_x - k_y)/2]a$ have different superconducting transition temperatures T_{c1} and T_{c2} ($< T_{c1}$), which results in a phase transition inside the superconducting phase at T_{c2} . A recently reported anomaly in the lower critical field measurements appears to support such exotic superconducting states (26).

Our results, together with the absence of a large magnetic ordered moment, impose constraints on theoretical models of the hidden order in URu_2Si_2 . The breaking of the four-fold rotational symmetry in a tetragonal crystal structure is in general a hallmark of electronic nematic phases (27). Electronic states that break crystal rotational symmetry without showing ordered moments have also been suggested in various strongly correlated electron systems, including $\text{Sr}_3\text{Ru}_2\text{O}_7$ (28), high- T_c cuprates (29), and frustrated magnets (30), which are discussed in terms of stripe or nematic orders. Our finding of a directional electronic state in a heavy-fermion material is another example of such exotic order in correlated matter.

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Figs. S1 to S3
References

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High-Gain Backward Lasing in Air

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The compelling need for standoff detection of hazardous gases and vapor indicators of explosives has motivated the development of a remotely pumped, high-gain air laser that produces lasing in the backward direction and can sample the air as the beam returns. We demonstrate that high gain can be achieved in the near-infrared region by pumping with a focused ultraviolet laser. The pumping mechanism is simultaneous resonant two-photon dissociation of molecular oxygen and resonant two-photon pumping of the atomic oxygen fragments. The high gain from the millimeter-length focal zone leads to equally strong lasing in the forward and backward directions. Further backward amplification is achieved with the use of earlier laser spark dissociation. Low-divergence backward air lasing provides possibilities for remote detection.

Optical techniques for the remote detection of atoms and molecules rely on the use of lasers to selectively identify and quantify species of interest. To enable single-sided detection, collection of light must be accomplished in the backward direction. Collection of incoherent light emission from molecules of interest is limited by the nondirectional nature of spontaneous emission. More sensitive detection techniques, aided by the coherent nature and well-defined direction of emission, are restricted in the direction of emission by the phase-matching relation. For commonly employed nonlinear tech-

niques such as coherent anti-Stokes Raman spectroscopy (1) and stimulated Raman scattering (2), phase-matching results in a coherent beam propagating in the direction of the pumping laser, away from the source.

These limitations have motivated the exploration of backward air lasing and stimulated gain concepts, which can produce coherent scattering that returns to the pump-laser location (3). To date, the only approach that has shown promise is based on the electron recombination of ionized molecular nitrogen from a femtosecond-produced filament (4, 5). This scheme leads to gain at 337 nm, the same wavelength as the molecular nitrogen laser. Amplified spontaneous emission gain on the order of 0.3 cm^{-1} has been observed (5).

We demonstrate the generation of high-gain lasing in air with the use of a 100-ps remote pump laser, which simultaneously drives a two-photon dissociation of molecular oxygen and a

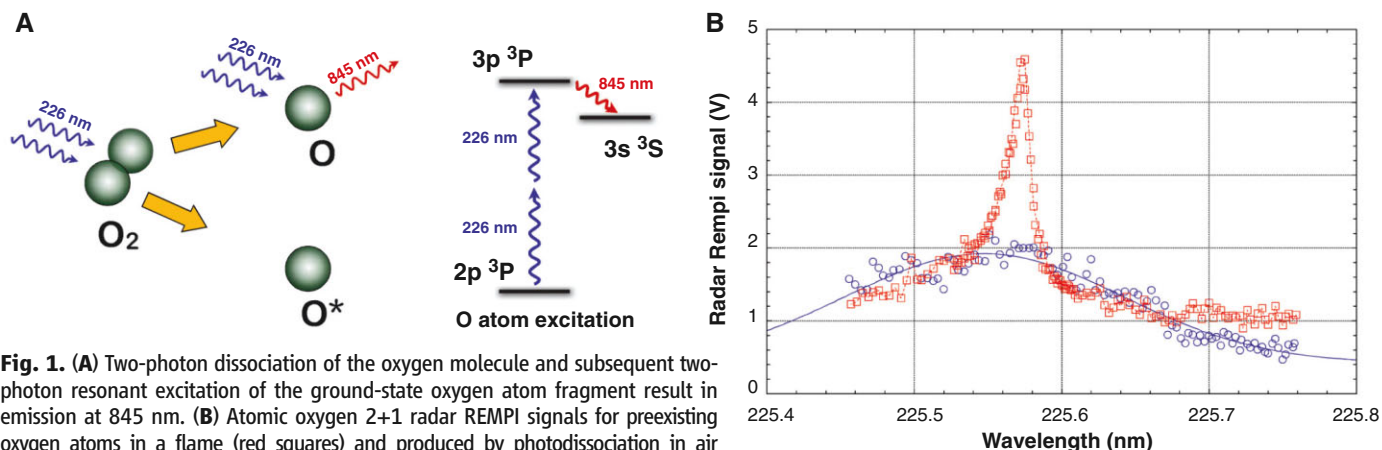
two-photon excitation of one of the resulting oxygen atom fragments. Both processes are resonantly enhanced at the 226-nm wavelength of the pump laser. The excitation is followed by lasing from the excited atomic oxygen (Fig. 1A). The pump laser is focused such that there is no laser-induced breakdown of the air, and excitation followed by stimulated emission is achieved throughout the 1-mm-long focal region. The result is the formation of well-collimated backward and forward propagating laser beams at 845 nm with parameters corresponding to the ultraviolet (UV) pump-beam focusing.

Two-photon laser-induced fluorescence from atomic oxygen has been developed for quantitative diagnostics of combusting gases where atomic oxygen is an important radical species (6–10). The two-photon excitation transition is from the $2p^3P$ ground state to the $3p^3P$ excited state with 226-nm laser radiation. That excitation is followed by spontaneous relaxation from the $3p^3P$ state to the $3s^3S$ state, producing fluorescence emission at 845 nm (Fig. 1A). The use of the two-photon excitation to produce stimulated emission at 845 nm in atomic oxygen has been observed in flames at subatmospheric pressures (11).

The same two-photon transition can be used as the initial step in a 2+1 resonance enhanced multiphoton ionization (REMPI) (12). This process can be remotely monitored by microwave scattering from the free electrons [radar REMPI (13)]. Figure 1B shows the radar REMPI excitation spectra from a flame containing oxygen atoms (squares) and from ambient air (circles) with a 100-ps laser tuned in wavelength through the two-photon oxygen atom transition at 226 nm.

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The 100-ps laser pulse is short enough to suppress avalanche breakdown (14), so multiphoton ionization of atomic oxygen dominates, and the measured radar REMPI signal reflects the density of atomic oxygen in the focal region of the laser. Note that in room air, even in the absence of the flame, the radar REMPI signal peaks at the same wavelength as the resonant atomic oxygen transition. The fact that this is not coincident with the resonant peak of the molecular oxygen ionization (15) indicates that the laser has caused the dissociation of molecular oxygen, and the observed peak is due to atomic oxygen, broadened by the high velocity of the oxygen atom fragments and their collisions with surrounding molecules (16). Similar molecular oxygen dissociation and atomic oxygen excitation have previously been observed with nanosecond pulses using two-photon laser-induced fluorescence (8, 11). The high velocity of the fragments suggests that they are formed by two-photon, rather than single-photon, dissociation. This observation is consistent with the measurements of Buijsse *et al.*, who used a 2+1 REMPI time-of-flight ion-imaging scheme to measure molecular oxygen photodissociation mechanisms and atomic oxygen fragment velocities (17). Their results indicate that three dissociation pathways are present at 226 nm: (i) a quite weak single-photon dissociative path via the Herzberg continuum forming $O(^3P_2) + O(^3P_j)$ with 0.38-eV excess energy, (ii) a dominant two-photon dissociative path via a resonance at 225.66 nm with the predissociative $3d\delta^3\Pi_{0,1}$ ($v = 2$, where v is the vibrational quantum number) Rydberg state leading to $O(^3P_2) + O(^1D_2)$ with 3.91-eV excess energy, and (iii) a weak two-photon resonant pathway through the same Rydberg state leading to $O(^3P_2) + O(^3P_j)$ with 5.88-eV excess energy. The dominant resonant two-photon pathway produces $O(^3P_2)$ atoms with velocities on the order of 4800 m/s.

The observation that the 100-ps laser pulse at 226 nm can simultaneously dissociate the molecular oxygen to ground-state atoms and excite the fast-moving oxygen atoms led to the realization that lasing might be possible in room-

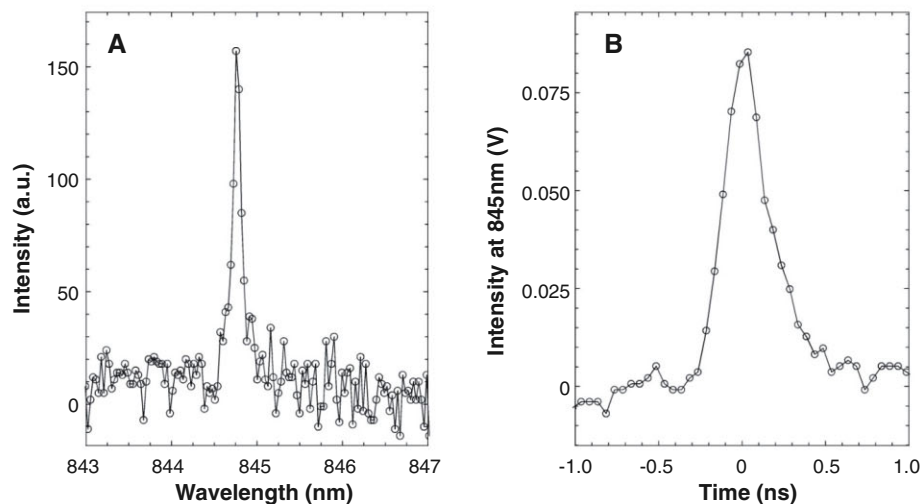


Fig. 2. Backscattered atomic oxygen emission (A) measured spectrum and (B) pulse in time. a.u., arbitrary units.

temperature, atmospheric-pressure air. The experimental setup is arranged to measure the gain and lasing properties in the backward direction, toward the pump source. The backward lasing is separated from the oppositely propagating pump by a dichroic filter and monitored by a detector, a fast-gated charge-coupled device (CCD) camera, or a spectrometer. The 10-Hz tunable 226-nm pump-laser pulses are obtained by mixing the output of a picosecond amplified tunable Ti:Sapphire laser with Nd:yttrium-aluminum-garnet (YAG) pulses and further frequency doubling. The 100-μJ pulses are focused into air with a 30-cm-focal-length lens, leading to a two-photon excitation volume of ~10-μm diameter and 1-mm length. The estimated focused peak intensity of 300 GW/cm² is low enough to ensure that the photodissociation process takes place without measurable ionization or spark formation within the 100 ps of excitation, so the pump laser is not scattered by plasma effects, and the gain path length of the excited atomic oxygen reflects the focal depth of the pump laser.

Figure 2 shows the measured spectrum and temporal pulse shape of the backscattered emis-

sion from atomic oxygen at 845 nm. The atomic oxygen spectrum shown in Fig. 2A is limited by the 0.1-nm resolution of the spectrometer. The 3-GHz oscilloscope used to measure the backscattered pulse duration in Fig. 2B indicates an upper limit of 300 ps for the atomic oxygen emission. The fact that the backward emission pulse width is more than two orders of magnitude shorter than the reported atomic oxygen fluorescence lifetime of 36 ns (11) is one indication of stimulated emission.

To establish the presence of lasing, the signal detected along the axis of the volume is compared to the signal detected when observing from the side. Because both approaches observe the same excited volume and collect the same solid angle, if the signal is incoherent fluorescence, the two measurements should give similar results. On the other hand, if the signal is due to stimulated emission, then there is exponential gain and the path length is important. In that case, the emission from the volume along the axis will be substantially stronger than the off-axis emission. Using a photomultiplier tube, we measure a ratio of more than 8000 between the emission

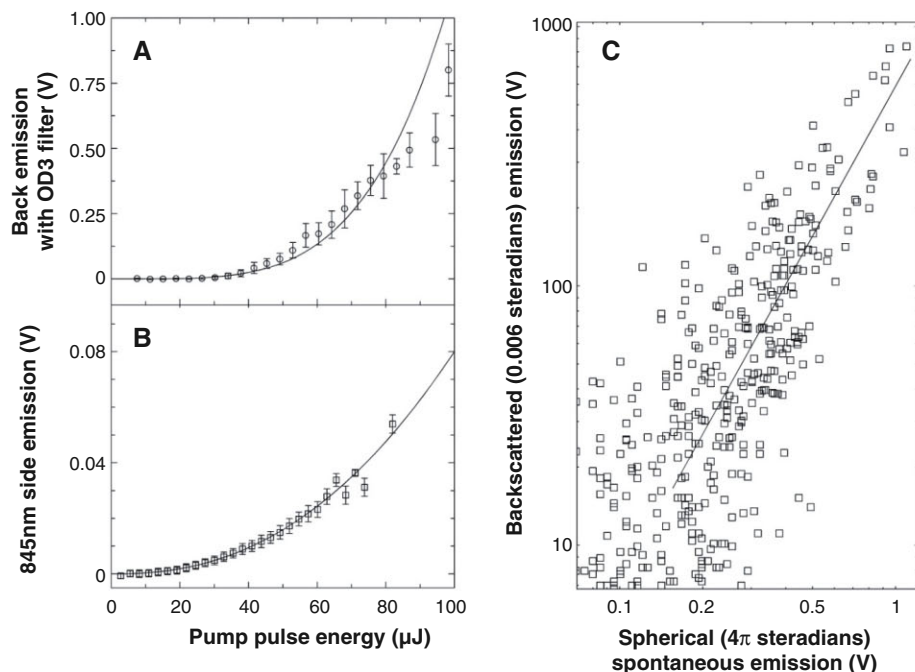


Fig. 3. The backward (A) and side (B) 845-nm emission in air as functions of the 226-nm pump-laser energy show the scaling difference between backward lasing (4th power) and incoherent emission (2nd power). Error bars indicate one SD. The backward coherent emission versus the total nondirectional incoherent emission (C) shows the stimulated emission gain.

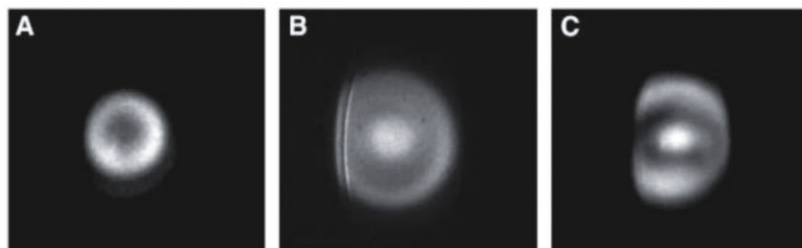


Fig. 4. Far-field, single-pulse backscattered oxygen laser beam at 845 nm measured 30 cm away from the 226-nm laser focused in (A) air, (B) a methane-air flame, and (C) air with a 532-nm pre-pulse-generated spark on the far side of the lasing region.

collected within the same 0.78-steradian (sr) solid angle along the axis in the backscattered direction and that collected from the side (Fig. 3, A and B), indicating that the backscattered emission is strongly gain-dominated.

Furthermore, if the emission were primarily incoherent, the scaling of the detected emission with the input UV pump-laser energy would be independent of the direction of observation. Figure 3 shows that the scaling is dramatically different. The axial signal (A) scales as the 4.2 power of the pump laser and has a threshold value, whereas the side-view signal (B) scales as the 2.3 power of the pump laser and has no threshold. From experiments at lower intensity with nanosecond laser pumping (11), we would expect the two-photon fluorescence scaling to have a power dependence of 3 or 4, depending on the route of dissociation of O_2 (one or two photons in addition to the two for excitation). The 2.3 power scaling shown in Fig. 3B implies strong saturation of either the dissociation or

excitation process. In a methane/air flame, where atomic oxygen is naturally present, we observe a subquadratic power dependence, indicating strong saturation of the two-photon excitation process. This suggests that in air, where the atomic oxygen density is smaller than in the flame, the two-photon excitation of atomic oxygen is saturated, whereas the dissociation of molecular oxygen retains a higher-order dependence.

Furthermore, in Fig. 3C, a direct comparison between the backward emission and side emission (while varying the pump power) shows a nonlinear dependence with the power of 2. As the voltage measurements of the two photomultipliers can be directly related to the respective signal amplitudes, we can also estimate the optical gain seen by the axial beam due to the stimulated emission process (Fig. 3C): The backscattered beam (which can be all collected in a 0.006-sr solid angle) is about 500 times stronger than the overall incoherent emission (obtained by extending the 0.78-sr side-collection

efficiency over the whole 4π solid angle). This corresponds to an optical-gain coefficient of 62 cm^{-1} over the 1-mm path length.

An overall signal gain in the backscattered direction can be estimated by using the ratio of the laser energy to the energy of the fluorescence collected into the same solid angle as the laser beam ($6 \times 10^{-3}\text{ sr}$). The solid angle ratio, multiplied with the optical gain of 500 times the energy increase of the axial beam when compared to the total fluorescence, leads to an increase on the order of 10^6 along the 1-mm axial path length of the excitation volume. Similar gain is measured in the forward direction.

As a final confirmation of lasing, Fig. 4 shows the single-shot, far-field images recorded 30 cm away in the backward direction by a nonamplified Princeton Instruments (Trenton, New Jersey) CCD camera. Figure 4A shows the image of the backward-propagating lasing in air. The beam is well localized, and the divergence of 40 mrad is consistent with diffraction-limited lasing from the cross-sectional size of the pump volume. The measured energy of the atomic oxygen laser pulse obtained in air for this focusing geometry is $\sim 20\text{ nJ}$, which provides an overall 2×10^{-4} efficiency for the backward-generated air laser.

The observed donut-shaped mode arises from the interaction of the pump laser with molecular oxygen. The presence of preexisting atomic oxygen generated by other means leads to a much stronger signal and produces a mode with a central maximum. For example, Fig. 4B shows the image of backward lasing from naturally occurring atomic oxygen in an atmospheric-pressure, stoichiometric methane/air flame, whereas Fig. 4C shows the atomic oxygen lasing from air when a spark is induced a few microseconds earlier by a 532-nm pulse from a frequency-doubled Nd:YAG laser. Using this pre-pulse to create atomic oxygen enhances the coherent backscattered emission by a factor of ~ 50 . For that enhancement to be effective for standoff applications, the spark must be located just on the far side of the lasing volume to minimize the distortions of the backward-propagating laser beam induced by the high-temperature spark region.

The optical gain and directional emission of the coherent backward radiation from the remote air laser open new opportunities for the standoff detection of atmospheric contaminants and trace species through stimulated and nonlinear backward scattering processes and also provide a much greater range for the detection of optical molecular and atomic features from a distant target.

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Magnetic Bistability of Molecules in Homogeneous Solution at Room Temperature

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Magnetic bistability, as manifested in the magnetization of ferromagnetic materials or spin crossover in transition metal complexes, has essentially been restricted to either bulk materials or to very low temperatures. We now present a molecular spin switch that is bistable at room temperature in homogeneous solution. Irradiation of a carefully designed nickel complex with blue-green light (500 nanometers) induces coordination of a tethered pyridine ligand and concomitant electronic rearrangement from a diamagnetic to a paramagnetic state in up to 75% of the ensemble. The process is fully reversible on irradiation with violet-blue light (435 nanometers). No fatigue or degradation is observed after several thousand cycles at room temperature under air. Preliminary data show promise for applications in magnetic resonance imaging.

Bulk materials exhibit magnetic properties fundamentally different from those of isolated atoms or molecules, as magnetism on the macroscopic scale is a collective phenomenon involving the mutual cooperation of large numbers of microscopic magnetic moments. A particularly important macroscopic property, generated by cooperative effects in the solid state, is magnetic bistability, or hysteresis, as found in the magnetization of ferromagnetic materials and spin crossover (SCO) complexes. Unfortunately, the maximum temperature at which the magnetic states are stable decreases with decreasing number of interacting atoms or molecules. This limitation has generally restricted technical applications at room temperature to particles of 7 to 10 nm and larger.

In contrast to ferro- and ferrimagnetic systems, where the magnetically interacting spins are located in neighboring atoms, the SCO effect provides control over the interaction of electron spins within a single atom (1). The phenomenon is observed in first-row transition metal complexes (d^4 to d^7 orbital occupancy). Depending on the nature and field strength of the surrounding ligands, the metal ion may exist in two different electronic configurations, low spin and high spin, which respectively minimize and max-

imize the number of unpaired d-electrons. If (for example, for intermediate ligand field strengths), both configurations are of similar energy, external perturbations such as temperature, pressure, or light can be used to switch between the two magnetic states (2). However, single molecules in solution undergo a gradual spin crossover following a Boltzmann distribution. Upon reversal of the external stimulus, the system immediately returns to the original state. In solids, cooperative effects (lattice effects) (3) may give rise to a hysteresis and thus to bistable behavior. For technical applications, the low-spin and high-spin states should be stable for a reasonable period of time within a large temperature range close to room temperature. It has been estimated that crystals with cooperative interaction of a minimum of a few thousand molecules are needed to meet these criteria (4). Temperature- and light-induced excited spin-state trapping (LIESST) in bulk crystalline materials has recently been demonstrated at ambient temperatures (5–8). However, these SCO systems exhibit bistability only in the solid state.

We herein demonstrate that, based on visible light-mediated reversible ligand coordination, the switching of magnetic properties can be performed at room temperature in magnetically non-interacting molecules in homogeneous solution. Hysteresis is achieved without cooperative effects, such as magnetic coupling (e.g., in ferromagnetic materials) or lattice interactions (e.g., in spin crossover systems).

Our technique is based on the idea that the spin state of a transition metal ion can be controlled by switchable ligands. In principle, there

are three approaches toward this end: varying the ligand field strength, switching the coordination number, or switching the degree of anti-ferromagnetic coupling between two high-spin metal ions (Fig. 1). The first approach, coined light-driven ligand-induced spin change (LD-LISC) has been investigated by multiple research groups using iron(II) (9–16) and iron(III) (17) complexes. A disadvantage of this concept is that the change in ligand field strength induced by external stimuli tends to be small and a complete switching between magnetic states over a wide temperature range is difficult to achieve. In contrast to the ligand field strength, the coordination number is a discrete, rather than smoothly variable, property, and the resulting magnetic states should be more robust to changes in the environment, such as temperature variations. We therefore pursued this approach for the design and synthesis of a prototype system. The tuning of antiferromagnetic coupling (Fig. 1C) is probably more difficult to achieve in transition metal complexes.

Nickel(II) is known to form complexes in two different spin states depending on the coordination number ($n = 4, 5$, and 6) (18). In square planar complexes ($n = 4$), Ni^{2+} tends to be low spin (diamagnetic, $S = 0$). Octahedral complexes ($n = 6$) are nearly always high spin (paramagnetic, $S = 1$). Square pyramidal complexes ($n = 5$) are either high or low spin depending on the nature of the ligands. Controlled coordination of two axial ligands to a square planar Ni^{2+} complex (i.e., modulation of the coordination number from 4 to 6) thus leads to a spin change from low ($S = 0$) to high ($S = 1$) spin. A simultaneous addition and removal of two ligands is difficult to

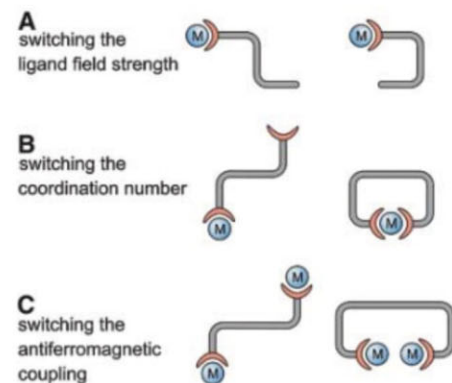


Fig. 1. (A to C) Three principles for ligand-driven spin-state change.

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realize in a robust molecular framework. We therefore performed preliminary investigations to find suitable organonickel systems that switch the spin state on changing the coordination number between $n = 4$ and 5 (19). Square planar Ni-tetrakis(pentafluorophenyl)porphyrin combined with pyridine as the axial ligand meets the criteria. To drive the coordination/decoordination processes of the axial ligand, we took advantage of the photochromic properties of phenylazopyridine (PAP). As with azobenzene, ultraviolet (UV) light irradiation (typically 365 nm) of *trans*-PAP induces isomerization to the *cis* configuration, whereas the back reaction is induced by visible light (435 nm) (20). This reversible switching process is accompanied by a large geometry change that in a well-designed molecular framework can be used to control coordination (21–23) and spin state [light-driven coordination-induced spin-state switching (LD-CISS)]. Hereby, the PAP unit serves both as a movable arm and as the axial ligand (24), thus keeping the system structurally simple. As in a macroscopic record player, the pickup arm (PAP) with the needle (lone pair of pyridine nitrogen) has to be connected to the turntable housing (Ni-porphyrin) in such a way that upon operation the needle is precisely manipulated to a well-defined position on the disk. In our case, the lone pair of the pyridine should be placed exactly perpendicular to the porphyrin plane and at a distance of 2.1 Å above the Ni^{2+} ion. There are three possible regioisomers of PAP (*o*, *m*, and *p*), two points of attachment to the porphyrin (β or *meso*) and numerous tethers for connecting the PAP arm with the porphyrin. We checked a large number of potentially suitable molecular structures and optimized their geometry by density functional theory (DFT) calculations. The target parameter for finding the most promising candidate was the distance between the pyridine nitrogen and Ni^{2+} ion. Ni-azoporphyrin 1 (Fig. 2) was selected for synthesis. DFT calculations (Perdew-Burke-Ernzerhof/Triple Zeta Valence Polarization) (25) predicted that the 1-*cis* isomer would be paramagnetic (triplet ground state) with an axial N-Ni bond of 2.086 Å, and 1-*trans* diamagnetic (singlet ground state) with an N-Ni distance of 6.350 Å (or larger depending on the conformation) (figs. S1 and S2).

The azo-functionalized porphyrin 1 was synthesized in a six-step sequence (26) (fig. S3). The key step was cyclization of phenyl dipyrromethane, PAP-functionalized benzaldehyde and pentafluorobenzaldehyde to form the metal-free monosubstituted tetraphenyl porphyrin. Ni(II) was inserted in the final step, and the resulting nickel porphyrin 1 was obtained in the thermodynamically more stable *trans* configuration. Upon irradiation of a dilute solution (~ 1 mM) of 1-*trans* in acetonitrile with bluish-green light (500 nm) at room temperature, the paramagnetic *cis* isomer is formed in $\sim 65\%$ yield (photostationary equilibrium). Thus, the wavelength inducing the *trans*-*cis* isomerization in 1 is red-shifted by about 135 nm as compared with most azobenzenes and azopyr-

ridines (~ 365 nm). We attribute this bathochromic shift to an energy transfer from the π, π^* excited state of the porphyrin (Q band) to the π, π^* state of the azopyridine. The isomerization is accompanied by a striking downfield shift and broadening of the nuclear magnetic resonance (NMR) signals of the protons in the vicinity of the paramagnetic Ni^{2+} center (Fig. 3, table S1, and figs. S4 to S7). We attribute these spectral changes to a combination of Fermi contact (contact shift) and dipolar couplings (pseudocontact shift) interactions between various nuclei in the molecule with the unpaired electrons in the nickel (27, 28). The pyrrole protons shift from 9.0 and 9.1 parts per million

(ppm) in 1-*trans* to 42.3, 43.2, and 43.7 ppm in 1-*cis*. The largest downfield shift is observed for the *o*-pyridine protons, which give rise to broad signals at 100 and 108 ppm.

Because of the paramagnetism of 1-*cis* we were not able to use nuclear Overhauser experiments (NOE) to obtain more detailed structural information. We therefore synthesized the analogous Zn-porphyrin from the metal-free precursor. The Zn complex exhibits an analogous *cis*-*trans* switching behavior to the Ni-porphyrin 1; however, it remains in the diamagnetic spin state. We isolated the *cis* and the *trans* configuration in pure form and unambiguously eluci-

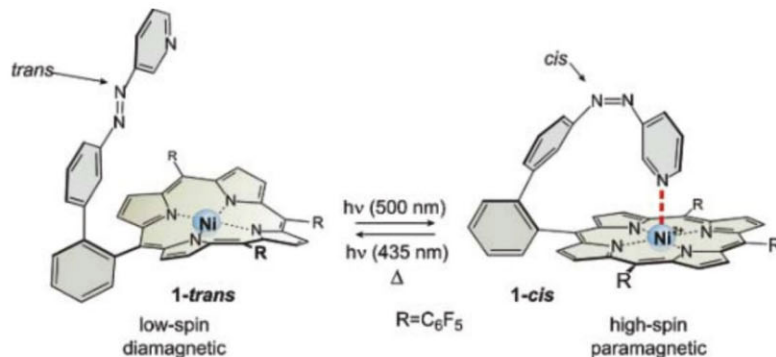


Fig. 2. Reversible light-induced magnetic switching of azopyridine functionalized Ni-porphyrin 1. Thermal conversion of the paramagnetic *cis* compound to the thermodynamically more stable *trans* isomer at room temperature in the dark is very slow. The half-life of 1-*cis* at 54°C in acetonitrile is 27 hours. The thermal stability in DMSO is even higher.

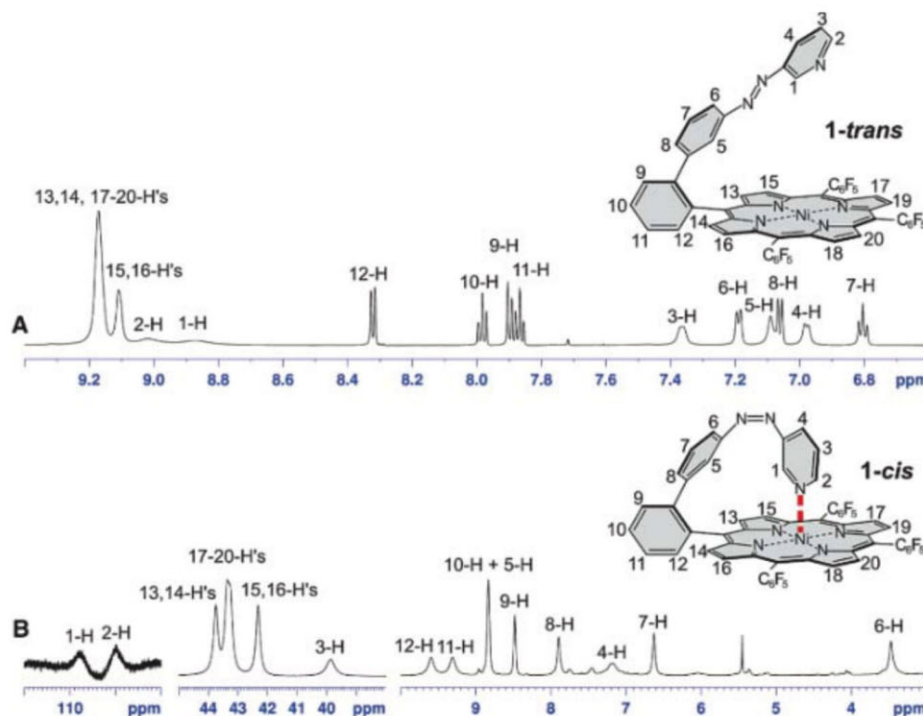
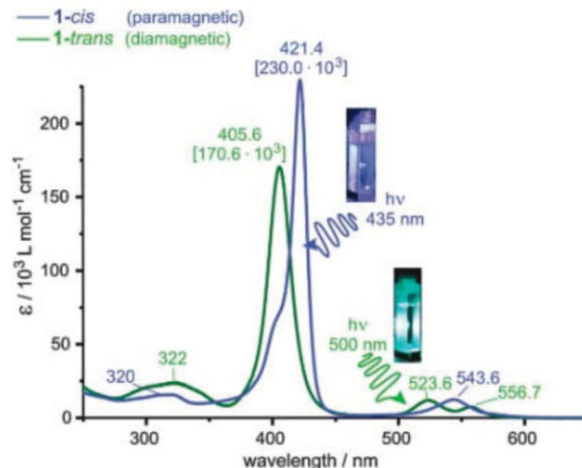


Fig. 3. $^1\text{H-NMR}$ spectra of the *trans* (A) and *cis* (B) configurations of porphyrin 1. The pyrrole protons (13 to 20) and, in even more pronounced fashion, the pyridine protons (1 and 2) in 1-*cis* exhibit a very large downfield shift and line broadening caused by magnetic coupling with the paramagnetic nickel center. The latter protons resonate at 100 and 108 ppm.

Fig. 4. UV spectra of the trans (green) and cis (violet-blue) configuration of Ni porphyrin 1. The $\lambda_{\max}$ for each absorption band is marked, and the extinction coefficients of the $\lambda_{\max}$ of Soret bands of 1-*cis* and 1-*trans* are given in brackets.



dated the structure by quantitative NOE experiments and DFT calculations (for details, see tables S4 to S6 and figs. S16 to 21).

In the UV-visible absorption spectrum during isomerization of 1-*trans* to 1-*cis*, the Soret band of the square planar Ni porphyrin ($\lambda_{\max} = 406$ nm) and the Q bands at 524 and 557 nm decrease in intensity, whereas new bands appear at $\lambda_{\max} = 422$ and 544 nm (Fig. 4). The cis isomer is extremely light sensitive and converts back to the diamagnetic trans species by irradiation with violet-blue light (435 nm) or by exposure to diffuse ambient light (photostationary states at different wavelengths are shown in fig. S11). The unusually high photoreactivity of 1-*cis* might be due to the fact that paramagnetic nickel(II) porphyrins release their axial ligands upon irradiation into the Soret band (29–31). The switching efficiency is even higher in dimethyl sulfoxide (DMSO). Upon irradiation at 500 nm, a photostationary equilibrium of 75% cis isomer is obtained. Irradiation at 435 nm switches the ensemble back to 97% of the trans form (3% cis).

The light-driven switching of magnetic properties in 1 is fully reversible, and the compound is completely stable under ambient conditions. Two samples of Ni porphyrin 1 (0.5 mL, ~50 μ M in CD₃CN in an NMR tube, and 3 mL, ~5 μ M in CH₃CN in a UV cuvette) were alternately irradiated with light-emitting diodes emitting light of 500 nm for 90 s (conversion to 45 and 50% 1-*cis*) and 435 nm for 30 s (conversion to 80 and 90% 1-*trans*) (fig. S12). No degradation or fatigue was detected after more than 10,000 cycles, even though the experiment was performed at room temperature under air (fig. S12). Particularly remarkable is the thermal stability of the cis isomer. Whereas *cis*-azobenzenes and *cis*-azopyridines typically isomerize to the more stable trans configuration with a half-life of several hours, 1-*cis* is stable for weeks (25% isomerization within 10 weeks at 21°C; half-life at 54°C, 27.2 hours) (figs. S13 and S14). The thermal stability of 1-*cis* is even higher in DMSO (<5% isomerization in 3 days at 70°C). Obviously, the cis configura-

tion is stabilized by coordination of the pyridine nitrogen to the Ni²⁺ ion.

Evans measurements were performed to determine the light-induced change in paramagnetic molar susceptibility (χ_M^p) of our switchable Ni-porphyrin 1 and to identify the effective magnetic moments (μ_{eff}) in the paramagnetic (1-*cis*), and the diamagnetic (1-*trans*) states (for details, see section VII in the supporting online material, fig. S15, and table S2). To prove that the magnetic switching arises from the proposed intramolecular isomerization, and not from intermolecular coordination, we performed the Evans measurements at different concentrations (0.73, 0.92, 1.34, and 1.95 mM) and showed the paramagnetic molar susceptibilities to be concentration independent ($\chi_M^p = 3749 \pm 19 \cdot 10^{-6}$ cm³ mol⁻¹) within the error limit of the method (table S2). From the measured χ_M^p values, the magnetic moment of pure 1-*cis* is $\mu_{\text{eff}} = 2.989 \pm 0.084$ B.M. (Bohr magneton). This value is well within the range found for paramagnetic ($S = 1$) Ni²⁺ complexes (2.8 to 3.4 B.M.) (18). The effective magnetic moment of 1-*trans* is found to vanish ($S = 0$) within the error limit of the method, in agreement with expectations.

As 1-*cis* corresponds to a pentacoordinate complex, the sixth coordination site is still available for further light-induced functions such as photoswitchable reactions (32), catalysis (33, 34), and controlled interaction with other spin systems (spintronics) (35). Further applications are possible because porphyrins are known to form ordered self-assembled monolayers and therefore are suitable to build nanometer-scale complex architectures (36, 37). Most interesting probably are applications that are possible because the paramagnetism of a solution now can be controlled by light with high spatial resolution. Complexes of paramagnetic ions are used as contrast agents in magnetic resonance imaging (MRI) (38). Switching a contrast agent on (high-spin state) and off (low-spin state) opens avenues for dynamic MRI (39). We performed preliminary studies in a commercial MRI scanner (Philips

Achieva 3.0 Tesla). A solution of nickel-porphyrin 1 (3.14 mL, 1.8 mM in DMSO) was irradiated with bluish-green light (500 nm, 75% conversion to 1-*cis*), and with white light (70% conversion to 1-*trans*). We observed a contrast difference (due to the change in relaxation time) of 43%, which corresponds well to the change in concentration of the paramagnetic 1-*cis* (45%) (fig. S22).

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Supporting Online Material

www.sciencemag.org/cgi/content/full/331/6016/445/DC1
 Materials and Methods
 SOM Text
 Figs. S1 to S22
 Tables S1 to S6
 References

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Kinetic Isotope Effects for the Reactions of Muonic Helium and Muonium with H_2

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The neutral muonic helium atom may be regarded as the heaviest isotope of the hydrogen atom, with a mass of ~ 4.1 atomic mass units (^4H), because the negative muon almost perfectly screens one proton charge. We report the reaction rate of ^4H with $^1\text{H}_2$ to produce $^4\text{H}^1\text{H} + ^1\text{H}$ at 295 to 500 kelvin. The experimental rate constants are compared with the predictions of accurate quantum-mechanical dynamics calculations carried out on an accurate Born-Huang potential energy surface and with previously measured rate constants of ^0H (where ^0H is shorthand for muonium). Kinetic isotope effects can be compared for the unprecedentedly large mass ratio of 36. The agreement with accurate quantum dynamics is quantitative at 500 kelvin, and variational transition-state theory is used to interpret the extremely low (large inverse) kinetic isotope effects in the 10^{-4} to 10^{-2} range.

Isotopic substitution provides a widely used tool for mechanistic analysis of chemical reaction rates and is a test bed for fundamental theories of chemical kinetics (1). A key simplifying assumption in chemical dynamics is the Born-Oppenheimer (BO) approximation (2), which allows the potential energy surface (PES) that governs nuclear motion to be calculated independently of the motion of the nuclear masses. The BO approximation facilitates the calculation and interpretation of kinetic isotope effects (KIEs), defined as the ratio of rate constants for two reactions differing only in isotopic masses, which provide mechanistic information even for complex reactions. The largest conventional KIEs are associated with substitution of ^2H or ^3H for ^1H . However, by using muons, one can access KIEs corresponding to isotopic mass ratios much greater than even 3 (3–5), and here we present an example with the unprecedentedly large ratio of 36.4.

The lightest H atom isotope is muonium (Mu), in which an electron (e) orbits a positive muon (μ^+)

“nucleus,” which is 206 times heavier than an electron, so that Mu [^0H , 0.113 atomic mass units (amu)] behaves chemically like a very light H atom (unlike positronium, for instance). Here we label Mu as ^0H to emphasize its importance for kinetics as a pseudo-isotope of H. Because of its light mass, ^0H has proven to be an unusually sensitive probe of quantum effects on nuclear motion in reaction rates, both in terms of zero-point-energy (ZPE) shifts and in its propensity to manifest quantum tunneling (5, 6).

In contrast to Mu, the heaviest usable isotope of H can be created by using a negative muon (μ^-) to replace an electron in He to form He μ , with an atomic mass of 4.116 amu (7). Because the muon is much heavier than an electron, its 1s orbital is very close to the nucleus (mean radius of 0.2 pm), effectively screening one proton

charge, so that He μ may be considered a virtual isotope of H, and so we will label it ^4H as shorthand. [The decimal character of 4.1 is a reminder that this is not a conventional H isotope such as quadrupium, ^4H , or other isotopes up to ^7H (8), with lifetimes, when known, in the 0.1 to 1 zs range.] Here we report a measurement of the rate constant $k_{4,1}$ for reaction of this heaviest isotope of the H atom with diprotium, and we compare the results with theory.

It has been said (9) that “Explaining the $\text{H} + \text{H}_2$ reaction is without question the most important problem in chemical dynamics. . . . A successful theoretical description is crucial because the credibility of every other theoretical advance in the field rests with the solution to the $\text{H} + \text{H}_2$ problem.” For $^2\text{H} + ^1\text{H}_2$ and $^1\text{H} + ^2\text{H}_2$, theory and experiment converged in 2003 (10). Here we report the application of the same theoretical methods to the reactions of ^4H and ^0H with $^1\text{H}_2$, along with results for the new experiments on the former. Combining these with previous (11) experimental results for the rate constant $k_{0,11}$ for the reaction $^0\text{H} + ^1\text{H}_2$ allows a comparison of experiment and theory for this fundamental reaction over a mass ratio of 36.4. The theoretical results are based on an accurate PES and accurate quantum dynamics calculations so that—in contrast to the usual situation—the theoretical results for this system can be used as benchmarks to validate the experimental approach. We also report and compare calculations based on variational transition-state theory (12) (VTST); this method is approximate, but testing it is important because of its applicability to complex reactions for which accurate quantum dynamics are impractical (1, 13).

Muon beams can be produced with essentially 100% longitudinal spin polarization at a nuclear

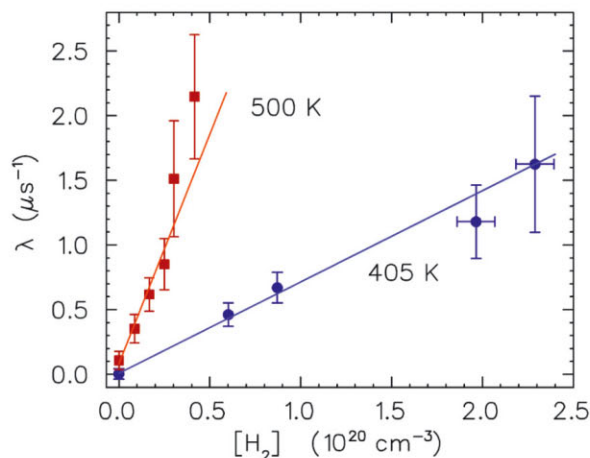


Fig. 1. Relaxation rates versus concentration of H_2 at 405 K (blue data points and fitted line) and 500 K (red data points and fitted line) at 500 bar of He.

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accelerator such as TRIUMF, where the present experiments with negative muons (μ^-) were carried out. In the decay of the muon ($\mu^- \rightarrow e^- \bar{\nu}_e \nu_\mu$), the resulting electron, which is detected in the experiments, is emitted preferentially opposite to the muon spin direction [see the supporting online material (SOM) (14)]. In a low transverse magnetic field, a fixed counter in the plane of precession will detect an oscillating signal with a characteristic Larmor frequency as the muon spin sweeps past its solid angle (15); this is the basis of the transverse-field muon spin rotation technique (16) we used.

Although the kinetic energies of muons are several million electron volts upon entering the gas target, most of this energy is lost to ionization and inelastic scattering processes down to energies of ~ 100 keV (16); there is no loss in muon spin polarization because the muon spin is unaffected by these Coulomb interaction processes. At lower energies, the μ^- is captured into a muonic orbit by He, which ejects both of its electrons, leaving the $^4\text{H}^+$ ion, which is neutralized in a charge-exchange collision (15) with ammonia dopant.

The experimental data are in histograms of the time differences between the detection of an incoming muon and the detection of its electron decay product. Superimposed on the signal is a function that reflects the interaction of the spin-polarized muon with its environment. Any process that causes a spin flip or spin dephasing causes a relaxation of the signal, with relaxation rate λ ; the most important process here is the pseudo-first-order reaction of ^4H with H_2 . The procedure for analyzing the data is presented elsewhere (7), and further experimental details are given in the SOM (14). Examples of the measured relaxation rates are in Fig. 1.

For high accuracy, the present calculations replace the usual BO PES with the Born-Huang (17) PES. The latter is obtained by adding the BO diagonal correction, which depends on nuclear masses. The BO surface (18) is a fit to essentially complete configuration interaction calculations. The diagonal correction (19) raises the BO barrier of 9.60 kcal/mol to 9.73 for ^4He and to 9.97 for $^{0.11}\text{H}$.

The quantum-dynamics calculations used the outgoing wave variational principle (20), numerically converged to better than 1%. The VTST calculations were carried out by improved canonical variational theory (ICVT) (21), with least-action ground-state (LAG) multidimensional tunneling contributions (22), and with the bound stretching vibration treated with the Wentzel-Kramers-Brillouin approximation (23) and bends treated with the centrifugal oscillator approximation (24).

The rate constants, $k_{4,1}$, determined from the slopes of plots such as those in Fig. 1, are shown in the Arrhenius plot of Fig. 2, where they are compared to theory. The VTST results are shown both with (ICVT/LAG) and without (ICVT) tunneling (both VTST results include ZPE); comparison of these results shows that tunneling makes a large contribution to the rate. The accurate quantum-mechanical (QM) calculations are in excellent agreement with the experimental data at

500 and 405 K, where they are well within experimental error, but the calculated rates are below experimental values at 295 K by 30%, a surprisingly large disagreement. Possible systematic errors that could be more important at the lowest temperature (where the rate is smallest) are impurities in the H_2 or additional relaxation caused by nonreactive collisions. The ICVT/LAG calculations of $k_{4,1}$ are consistently below both accurate QM theory and experiment at all temperatures, although they agree with the QM results within 23% over the temperature range plotted. The largest uncertainties in the ICVT/LAG calculations are in the tunneling contributions.

The present experimental results for the reaction rate of the heaviest H-atom isotope, muonic He, with the protium molecule can now be compared with that for the lightest H-atom isotope, muonium, corresponding to an unprecedented factor of 36.4 in atomic mass. The ratio of rate constants is given in Fig. 3, which contains only one experimental point because the $^{0.11}\text{H} + ^1\text{H}_2$ experiments (11) extend down only to 473 K. The usual convention is to place the rate constant for the lighter isotope in the numerator, because that yields a normal KIE greater than 1. The present KIE defined this way, $k_{0.11}/k_{4,1}$, however, is inverse (less than 1) and strikingly small, with an experimental value of 0.0108 and an accurate quantum value of 0.0104 at 500 K. The finding that accurate quantum dynamics agrees essentially perfectly with experiment, even though the KIE is a very strong function of temperature (Fig. 3), argues convincingly that ^4H is indeed a H isotope, and noth-

ing (such as geometric phases or nonadiabatic behavior) beyond a nonrelativistic treatment of single-potential-energy-surface scattering on the Born-Huang surface needs to be considered. This result is a dramatic confirmation of the success of quantum scattering calculations and the experiments in a difficult arena. When the temperature is lowered, the accurate quantum scattering result becomes 2.46×10^{-3} at 405 K and 1.74×10^{-4} at 295 K, with the latter being (to our knowledge) the smallest KIE ever reported.

Conventional transition-state theory without tunneling, which is widely used to interpret reaction mechanisms (25), predicts a KIE of 1/19 at 500 K, in contrast to 1/90 by ICVT/LAG and the accurate value of 1/96. The good agreement of VTST calculations with accurate quantum results allows them to be used to assess vibrational contributions at the transition state and the importance of quantum tunneling, neither of which is explicit in the fully quantum calculations. The VTST calculations show that the large inverse KIE may be attributed primarily to the difference in stretching-vibration ZPE at the two transition states (the two reactions have identical reactant ZPEs). At their respective variational transition states at 500 K, the ^4He reaction has a ZPE of 5.3 kcal/mol (2.9 in stretch and 2.4 in bend), whereas the $^{0.11}\text{H}$ reaction has a ZPE of 13.8 kcal/mol (9.8 in stretch and 4.0 in bend). The good agreement

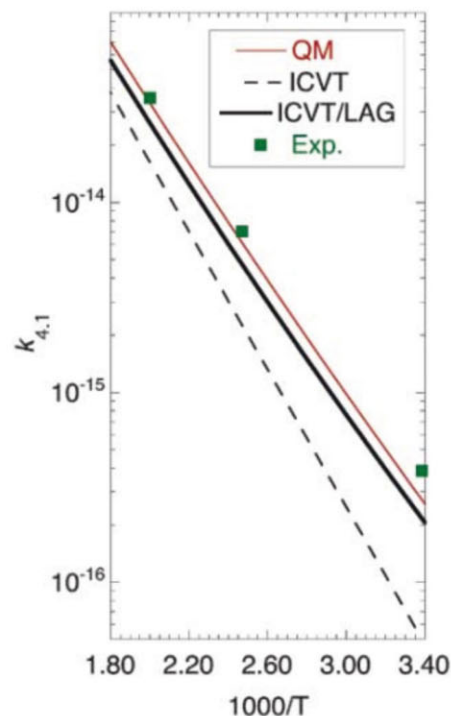


Fig. 2. Comparison of experimental and theoretical thermal rate constants of the $^4\text{H} + ^1\text{H}_2$ reaction (in units of cubic centimeters per molecule per second).

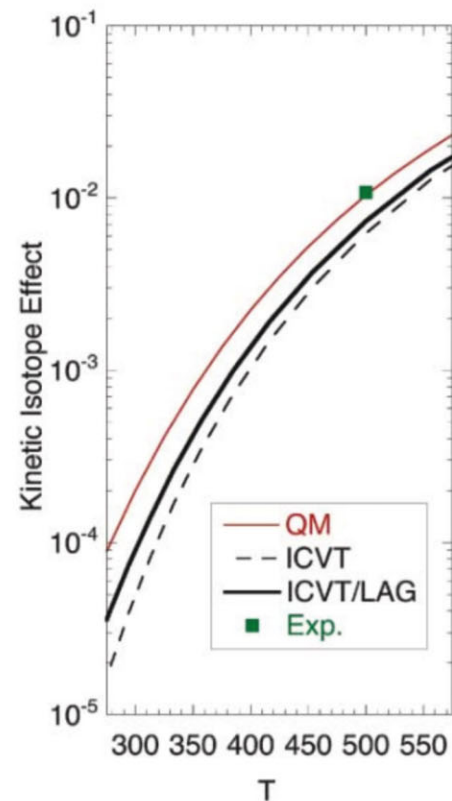


Fig. 3. Comparison of experimental and theoretical kinetic isotope effects ($k_{0.11}/k_{4,1}$). The experimental point was obtained using the published experimental fit for the $^{0.11}\text{H}$ reaction and the experimental datum at 500 K for the ^4H reaction.

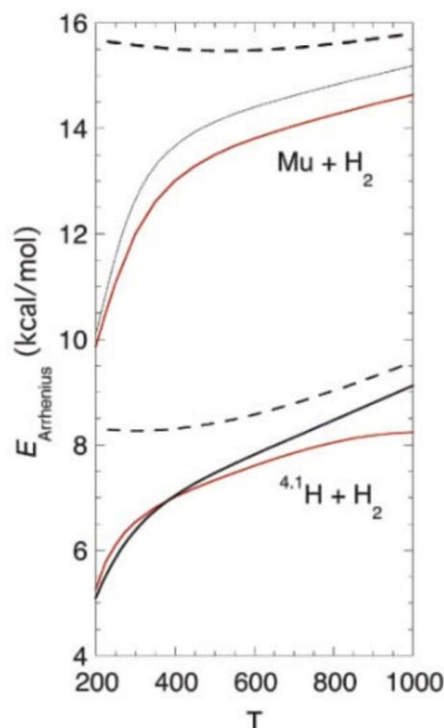


Fig. 4. Comparison of Arrhenius activation energies ($E_a = E_{\text{Arrhenius}}$) calculated via QM (red solid lines), ICVT (dashed black lines), and ICVT/LAG (solid black lines) for the reactions of $^4\text{H} + \text{H}_2$ and $^{0.11}\text{H} + \text{H}_2$ as a function of temperature (T).

(within 23%) of the VTST calculations with experiment lends credence to the existence of quantized transition states (26, 27) with these large amounts of vibrational energy.

The tunneling transmission coefficient, which is the ratio of the ICVT/LAG rate constant to the ICVT rate constant, is 4.3, 2.1, and 1.6 at 295, 405, and 500 K, respectively. Reactions of $^{0.11}\text{H}$ may exhibit greater tunneling effects than heavier isotopes because of the small mass (6), and this would be true in any one-dimensional treatment

with an isotope-independent effective barrier. However, the LAG approximation is multidimensional, including ZPE effects (28, 29) in the effective tunneling barriers and isotope-dependent tunneling paths, and the effective barrier to tunneling is much broader for the $^{0.11}\text{H}$ case because of the large ZPE of the $^{0.11}\text{H}^1\text{H}$ product (30). It is very encouraging that the results based on the LAG tunneling treatment, which is affordable for complex systems (31), correctly accounts for the KIE despite the quite different effective potentials for the two isotopes, confirming the physicality of the isotope-dependent barriers.

The Arrhenius (32) activation energy is defined as

$$E_a = -R \frac{d \ln k}{d(1/T)} \quad (1)$$

which is proportional to the negative slope of an Arrhenius plot. It is well known (33, 34) that E_a can exhibit substantial temperature dependence, and the present reactions illustrate this in Fig. 4; this kind of detail cannot be revealed yet by experiment. Figure 4 shows that E_a would be quite different in magnitude and temperature dependence without tunneling, but with tunneling, the ICVT/LAG calculation agrees very well with the dramatic temperature dependence and isotope dependence shown by the accurate quantum-dynamical results. These results confirm the usefulness of VTST for interpreting large quantum effects.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/331/6016/448/DC1

Methods

Scheme S1

References

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Enhanced Modern Heat Transfer to the Arctic by Warm Atlantic Water

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The Arctic is responding more rapidly to global warming than most other areas on our planet. Northward-flowing Atlantic Water is the major means of heat advection toward the Arctic and strongly affects the sea ice distribution. Records of its natural variability are critical for the understanding of feedback mechanisms and the future of the Arctic climate system, but continuous historical records reach back only ~150 years. Here, we present a multidecadal-scale record of ocean temperature variations during the past 2000 years, derived from marine sediments off Western Svalbard (79°N). We find that early–21st-century temperatures of Atlantic Water entering the Arctic Ocean are unprecedented over the past 2000 years and are presumably linked to the Arctic amplification of global warming.

Rising air temperatures (1–3) and a decline of the sea ice cover (4, 5) evidence a rapid warming in the Arctic that has

reversed a long-term cooling trend (6). Relatively warm Atlantic Water (AW) in the Fram Strait Branch (FSB) of the North Atlantic Cur-

rent is the major carrier of oceanic heat to the Arctic Ocean (Fig. 1). It maintains perennially ice-free conditions in the eastern Fram Strait today and supplies salt to the intermediate and bottom waters of the Arctic Ocean, thereby stabilizing the stratification (7, 8). In the eastern Fram Strait, AW with temperatures of 2° to 6°C and salinities of >35.0 is found at 50- to 600-m water depth (Fig. 2). Most of the year it is overlain by a mixed layer of lower salinity, seasonally variable temperatures, and ice coverage in ex-

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treme winters. These parameters also varied in the past 2000 years, as shown by sea ice observations and sediment core studies (5, 9).

Highly variable AW advection to the Arctic in the Late Quaternary has been recorded by proxies like microfossil abundances, flux rates, and species ratios (subpolar versus polar species) in sediment cores. Advection maxima occurred mostly during relatively warm periods (interglacials and interstadials) (10–12). The Holocene [past 12 ky (ky, thousand years)] was characterized by a thermal maximum at 10 to 9 ky and a cooling thereafter (13). Previous studies, however, were unable to clearly resolve variations within a few hundred years or less. Such varia-

tions are well known from historical and proxy data of European climate (14) and subdivide the last 2 ky into the Roman Warm Period (RWP, until ~600 CE), the Dark Ages Cold Period (DACP, ~600 to ~900 CE), the Medieval Climate Anomaly (MCA, ~900 to ~1500 CE), the Little Ice Age (LIA, ~1500 to ~1900 CE), and the Modern (Industrial) Period. Ages of boundaries between individual periods may vary regionally.

To reconstruct the temperature variability of AW in the FSB within the past ~2000 years, we investigated planktic foraminifers in a sediment core obtained in August 2007 from the western Svalbard continental margin at site MSM5/5-712 (Fig. 1). This site is strategically situated in

the path of AW inflow to the Arctic Ocean. Radiocarbon dates in core MSM5/5-712-1 (table S1) revealed sedimentation rates of 18 to 20 cm ky^{-1} for the period 120 BCE to 1475 CE and 27.7 cm ky^{-1} thereafter, resulting in a resolution of 28 and 18 years per 0.5-cm sample, respectively (Fig. 3). The core top had a modern age. Bioturbation by bottom-living organisms is expected to somewhat smooth paleoenvironmental signals, but a quantification of the effect is difficult. Our temperature reconstruction of AW inflow to the Arctic Ocean is based on two independent methods: (i) The SIMMAX modern analog technique (15) applied on planktic foraminifer species counts to calculate temperatures at 50-m water depth and (ii) Mg/Ca measurements on the species *Neogloboquadrina pachyderma* (sinistral). Method details are given in the Supporting Online Material (SOM). Habitat and calcification depth of planktic foraminifers in the eastern Fram Strait are below 50-m water depth and reach down to ~300 m, but the distribution maximum is above 150 m (16). In this area, plankton blooms usually occur in August (16). Thus, our temperature reconstructions reflect mid-summer conditions in the uppermost part of the AW layer, averaged over a few decades.

Planktic foraminifer associations are useful indicators of water mass distributions and oceanic fronts in the northernmost North Atlantic (17). The planktic foraminiferal data from core MSM5/5-712-1 reveal multicentennial changes underlying a high-frequency (multidecadal) variability (Fig. 3A; for details of species distributions, see fig. S1). High flux rates of both polar and subpolar species characterize the RWP, the MCA-LIA transition, and the Modern Period, whereas low fluxes are recorded for the DACP and the late LIA (Fig. 3A). These flux variations likely reflect sea ice cover, because lowest planktic foraminiferal fluxes are today found in the ice-covered areas of the Fram Strait, and highest fluxes at the ice margins (18). In sediments from before ~1900 CE, 10 to 40% of all planktic foraminifers belong to subpolar species. In contrast, the youngest sediments reflecting the past ~100 years show a steep increase in subpolar foraminifer fluxes and an unprecedented inversion of the subpolar/polar species ratio, reaching 66% subpolar specimens in the surface sample (Fig. 3B). Because the percentage of subpolar specimens is closely related to the water mass distribution, with low values in Arctic waters and high values in AW (16–18), highest subpolar foraminifer fluxes and percentages in samples from the Modern Period indicate a strongly increased influence of warm AW advected from the Norwegian Sea.

Results from SIMMAX and Mg/Ca measurements allowed us to quantify the temperature increase associated with the stronger influence of AW off Western Svalbard during the Modern Period. Until ~1850 CE, average summer temperatures varied between 2.8° and 4.4°C (mean $\pm$ SE: 3.4° $\pm$ 0.06°C) according to SIMMAX, with low values during the well-known cold periods

Fig. 1. Bathymetric map of the Fram Strait area and the eastern Arctic Ocean (inset; source: www.ibcao.org). Average sea ice coverage for April [1989 to 1995; stippled line: 1963 to 1969 (31)] and September (inset; 1979 to 2000; source: http://nsidc.org) is indicated by white shading. White arrows indicate ice flow direction in Fram Strait area. Red arrows indicate flow direction of Atlantic Water. Atlantic water flow is below halocline waters in the Arctic Ocean proper. Yellow spot marks station MSM5/5-712 at 78°54.94'N, 6°46.04'E, 1491-m water depth. BS, Barents Sea; LS, Laptev Sea.

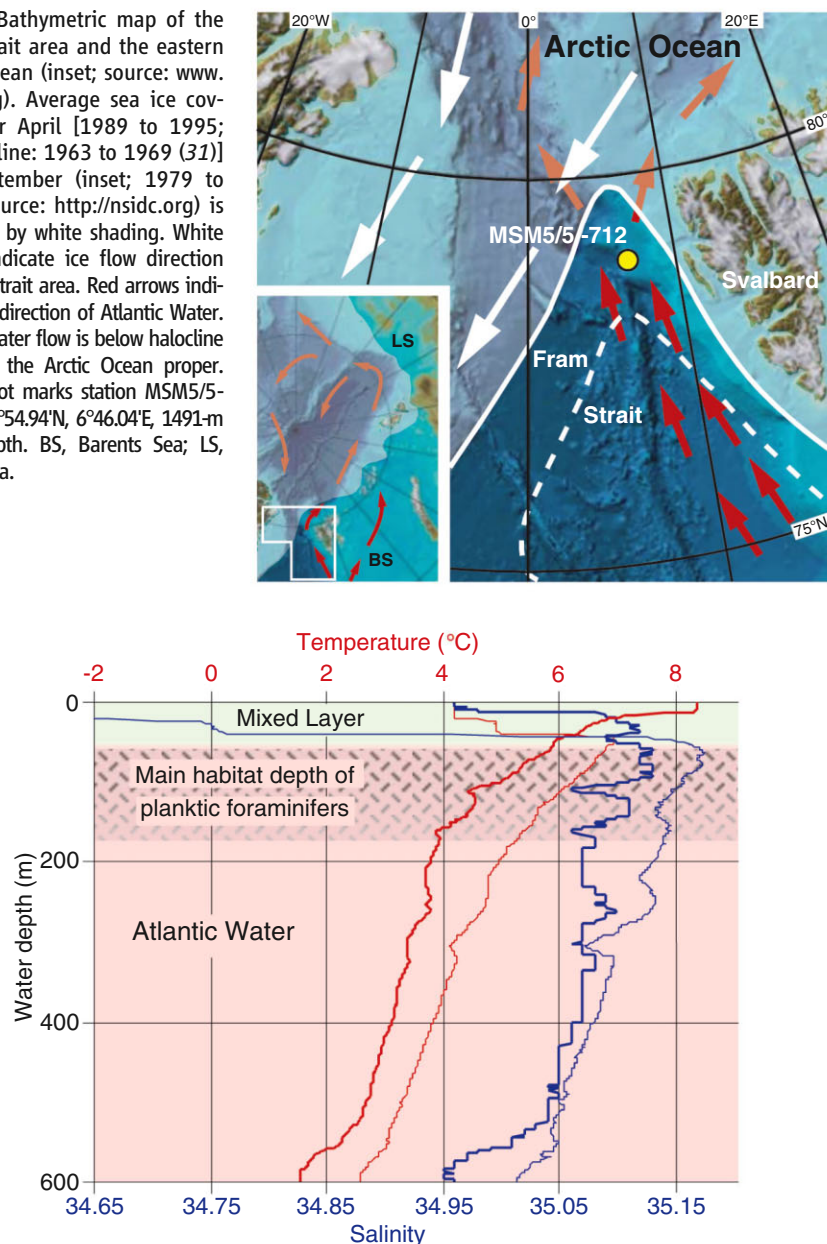


Fig. 2. Water-mass structure of the upper 600 m at station MSM5/5-712. Seasonal variability of temperature (red lines) and salinity (blue lines) in the Atlantic Water (pink) is revealed by our measurements in summer (4 August 2007; bold lines) and early winter (11 October 2006; thin lines). The main habitat of planktic foraminifers in the eastern Fram Strait (16, 18) is marked by a dashed pattern.

(early DACP, LIA) and maxima during the early MCA at 800 to 1100 CE (Fig. 3C). The record shows strong similarities to terrestrial records of Northern Hemispheric and circum-Arctic temperature variability (6, 19) (Fig. 3E). AW temperatures since 1890 CE were 4.1° to 6.0°C (mean $\pm$ SE: $5.2^{\circ} \pm 0.22^{\circ}\text{C}$) and thus $\sim 2^{\circ}\text{C}$ higher than during the previous 2 ky. The rapid increase to an unprecedented maximum of 6°C calculated for the surface sample apparently started already around 1850 CE. However, the gradual transition may be an artifact caused by bioturbation mixing of foraminifer-poor sediments from the late LIA and foraminifer-rich sediments from the Modern Period. Temperature reconstructions from Mg/Ca measurements give results very similar to those of SIMMAX (Fig. 3D). Variability before the Modern Period is high (0.7° to 5.7°C), probably because the Mg/Ca method could not precisely reproduce temperatures below 3°C [(20); see discussion in the SOM], but the temperature mean of 3.6°C ($\pm 0.3^{\circ}\text{C}$, SE) confirms the SIMMAX results. The same agreement applies for the Modern Period: Temperatures since 1890, as calculated from Mg/Ca data, range from 4.4 to 7.1°C with a mean value of 5.8°C ($\pm 0.5^{\circ}\text{C}$, SE). For both methods, the temperature mean of the Modern Period exceeds all individual values from the preceding 2000 years. These results reveal a rapid warming by $\sim 2^{\circ}\text{C}$ of uppermost AW in the FSB in the Arctic Gateway during the past ~ 120 years, consistent with the documented sea ice retreat in the Barents Sea (5), terrestrial paleoclimate reference records (6, 19) (Fig. 3, C to E), and atmospheric measurements (3). Notably, modern summer temperatures of uppermost AW in the eastern Fram Strait are $>1.5^{\circ}\text{C}$ higher than multidecadal mean temperature maxima (averaged by bioturbation and sampling) during the MCA.

Our reconstructed warming of $\sim 2^{\circ}\text{C}$ since the LIA matches the reported temperature increase of the Arctic Atlantic Water Layer (AAWL), obtained from observational data of the past ~ 120 years (21) (Fig. 3C). At present, there are no subcentennial-scale open ocean proxy data series available to document the temperature evolution of AAWL in the Arctic Ocean proper in the preceding two millennia. Further upstream, in the eastern Norwegian Sea, high-resolution records of summer sea surface temperature (SSST) variability from diatoms (22) and stable isotopes of planktic foraminifera (23) indicate a warming of $\sim 1.5^{\circ}\text{C}$ off western Norway since the LIA. However, eastern Norwegian Sea SSSTs in the late 1990s do not clearly deviate from those occurring periodically during the MCA (22, 23). Our finding of unequalled warm modern AW temperatures in the eastern Fram Strait with respect to the previous 2000 years (including the warm periods in Roman and Medieval times) may thus express another facet of the Arctic amplification (1, 24) of global warming. Recent model results (25, 26) reveal the important role of sea ice and atmospheric pressure fields in the Barents Sea as

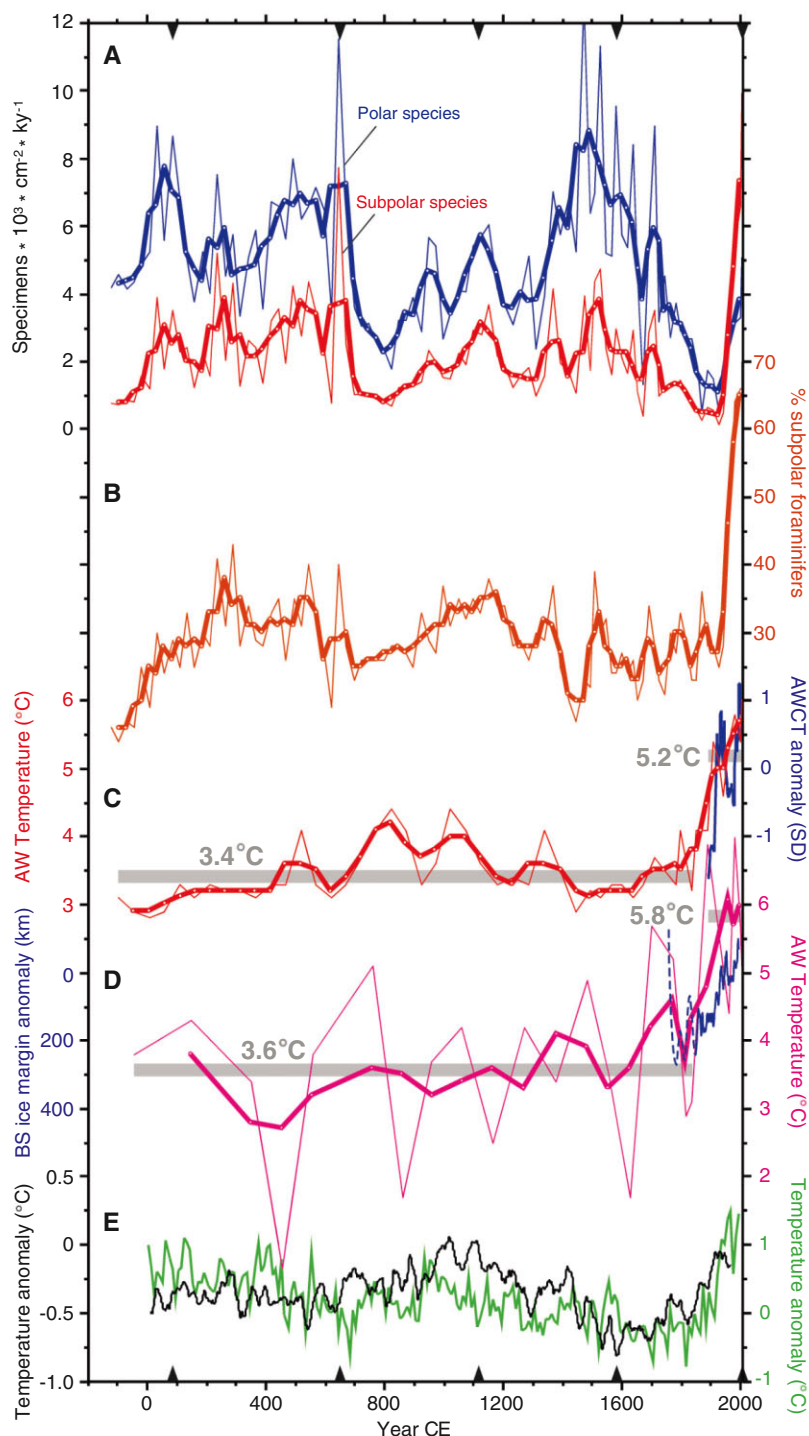


Fig. 3. Planktic foraminiferal data and temperature reconstructions of upper Atlantic Water in the eastern Fram Strait over the past ~ 2100 years from sediment core MSM5/5-712-1. Thin lines are raw data, bold lines are three-point running means. Black triangles on the age scale mark calibrated accelerator mass spectrometry ^{14}C ages. (A) Fluxes of polar and subpolar planktic foraminifera (100- to $250\text{-}\mu\text{m}$ fraction). (B) Percentage of subpolar planktic foraminifera in the 100- to $250\text{-}\mu\text{m}$ fraction. (C) Summer temperatures at 50-m water depth (red) calculated by the SIMMAX Modern Analog Technique. Gray bars mark averages until 1835 CE and 1890 to 2007 CE. Blue line is the normalized Atlantic Water core temperature (AWCT) record (standard deviations) from the Arctic Ocean (1895 to 2002; 6-year averages) obtained from (21). (D) Summer temperatures (purple) calculated from Mg/Ca ratios in planktic foraminifera *N. pachyderma* (sinistral). Gray bars mark averages until 1835 CE and 1890 to 2007 CE. Blue line is the sea ice margin anomaly (11-year means, less ice is up) in the Barents Sea (BS) obtained from (5). Dashed lines mark less reliable data before 1850 CE. (E) Terrestrial Arctic [green, from (6)] and Northern Hemisphere [black, 25-year means, from (19)] temperature anomaly records with reference to the 980 to 1800 CE and 1961 to 1990 CE averages, respectively.

a possible amplifier, which may, at least in part, be responsible for the exceptionally warm AW advection.

Instrumental air and AW temperatures in the Arctic during the 20th century and beyond display quasi-synchronous multidecadal oscillations that make isolation of the industrial warming trend difficult (3, 21). Basinwide observations since the 1980s detected multiyear events of AW spreading in the Arctic Ocean that featured both a strong warming and an increased inflow to the Arctic (7, 27, 28). Although we cannot quantify from our data the variability of previous AW inflow to the Arctic by volume, our temperature data series and the above observational link suggest that the modern warm AW inflow (averaged over two to three decades) is anomalous and unique in the past 2000 years and not just the latest in a series of natural multidecadal oscillations. Both effects—a temperature rise as well as a volume transport increase—introduce a larger heat input into the Arctic Ocean. Although there is no direct contact of the AAWL with the ocean surface in the Arctic, such an increased heat input has far-reaching consequences. The strong AW warming event in the Arctic Ocean in the 1990s caused a shoaling of the AW core and an enhanced heat flux to the surface (29), concurrent with decreasing sea ice (4). Recent oceanographic data from the Laptev Sea continental margin indicate the impact of warm AW-related water masses on the shallow (<50 m) shelf (30), a feature not observed before in a >80-year time series. The data also provide evidence for a significant heat flux to the overlying shelf waters (30). Even without any modification of the vertical heat transfer processes, the enhanced temperature contrast between the AW and the surface sea water freezing point (increased from

~5 to 7 K as identified here) leads to an increase in the vertical heat flux of ~40%. Any positive-feedback mechanism will magnify the effect of this flux increase on the ice cover. Complementing the strong feedback between ice and atmospheric temperatures (1), warming of the AW layer, unprecedented in the past 2000 years, is most likely another key element in the transition toward a future ice-free Arctic Ocean.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/331/6016/450/DC1

Methods

Fig. S1

Table S1

References

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The Southern Route “Out of Africa”: Evidence for an Early Expansion of Modern Humans into Arabia

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The timing of the dispersal of anatomically modern humans (AMH) out of Africa is a fundamental question in human evolutionary studies. Existing data suggest a rapid coastal exodus via the Indian Ocean rim around 60,000 years ago. We present evidence from Jebel Faya, United Arab Emirates, demonstrating human presence in eastern Arabia during the last interglacial. The tool kit found at Jebel Faya has affinities to the late Middle Stone Age in northeast Africa, indicating that technological innovation was not necessary to facilitate migration into Arabia. Instead, we propose that low eustatic sea level and increased rainfall during the transition between marine isotope stages 6 and 5 allowed humans to populate Arabia. This evidence implies that AMH may have been present in South Asia before the Toba eruption (1).

The deserts of the Arabian Peninsula have been thought to represent a major obstacle for human dispersal out of Africa. AMH

were present in East Africa by about 200 thousand years ago (ka) (2). It is likely that the first migration of AMH out of Africa occurred im-

mediately before or during the last interglacial [marine isotope stage (MIS) 5e] (3). During MIS 6, the Afro-Asiatic arid belt was hyperarid, restricting movements of human populations out of Africa. Finds from Qafzeh and Skhul in the Near East, dated between 119 ± 18 and 81 ± 13 thousand years ago (ka) (4, 5), suggest that AMH first migrated along the “Nile Corridor” and into the Levant. A later pulse from <65 to 40 ka is thought to have led to further colonization into Europe

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and Asia (6–8). An early southern dispersal from East Africa into south Arabia has been suggested as an alternative route out of Africa, drawing upon faunal and floral evidence (9), human genetics (10–12), and several Paleolithic surface occurrences (3), some of which were first described in the 1930s (13). Here, we describe evidence for the presence of AMH by about 125 ka at Jebel Faya (25.119°N 55.847°E, Fig. 1) (14). Jebel Faya is a 10-km-long, north-south-oriented limestone mountain outlier rising to ~350 m above sea level (m asl). It is located ~55 km from both the Gulf of Oman and the Persian Gulf and directly south of the Straits of Hormuz. At its closest, the present coast of Iran is 165 km away.

The archaeological site, named FAY-NE1, is a rock-shelter (180 m asl) at the northeastern end of Jebel Faya. Excavations at the site were undertaken between 2003 and 2010. A 24-m-long section, up to 5 m in depth, was cut from the back wall of the shelter outward (fig. S3). Several other trenches were opened within and in front of the shelter. An area exceeding 150 m² has been excavated. The site contains archaeological levels and artifacts that include the Iron and Bronze Ages, the Neolithic, and the Paleolithic. We used single-grain optically stimulated luminescence (OSL) dating (15) to determine the age of Paleolithic layers. The Holocene levels are separated from the Paleolithic layers by archaeologically sterile sediments. We deal here with the three Paleolithic assemblages designated as A, B, and C (16) because they do not conform to any known named Paleolithic industries. Assemblage A is the uppermost and is separated from the lower assemblages by sterile sediments. Assemblages B and C are not always well separated but are clearly superimposed upon one another.

Three OSL samples associated with assemblage C yielded ages of 127 ± 16 (1 SE uncertainties), 123 ± 10 , and 95 ± 13 ka (table S8). Lithics from assemblage C (Fig. 2) exhibit a number of different reduction strategies; blank production was by the Levallois, volumetric blade, and simple parallel methods. Typologically the tools include small hand axes, foliates, foliate preforms, end scrapers, sidescrapers, and denticulates (16).

Assemblage B blanks, mainly flakes and few blades, were largely produced from flat flaking surfaces with parallel, converging, and crossed removals. Tools include sidescrapers, end scrapers, denticulates, retouched pieces, burins, and perforators. Unlike assemblage C, there was no evidence for the Levallois technique, although there were some small volumetric blade cores. The number and range of tools suggests that the occupation was multipurpose.

Assemblage A contains mainly flakes struck from multiple platform cores with parallel removals on each face. Blades are rare. Tools include burins, retouched pieces, end scrapers, sidescrapers, and denticulates. Both assemblages A and B lack Levallois flaking and bifacial reduction, and, typologically, the use of backing was unknown. Assemblage A is overlain by ~40 cm of sterile

sand. Two OSL samples from within assemblage A yielded ages of 38.6 ± 3.1 and 40.2 ± 3.0 ka, and two samples from the overlying sterile layer yielded ages of 38.6 ± 3.2 and 34.1 ± 2.8 ka. Scattered early Holocene lithics, including Fasad points, were found immediately above the sterile sand layer. Two marine shells found in association with Fasad points yielded radiocarbon ages

of 10,405 to 9711 and 10,380 to 10,078 calendar years before the present (cal. yr B.P.), indicating early Holocene human occupation of the south-eastern Arabian Peninsula (17).

Assemblage C ages are consistent with those of the Levantine Middle Palaeolithic, but the assemblage is neither technologically nor typologically related to that of the Levant. Notably

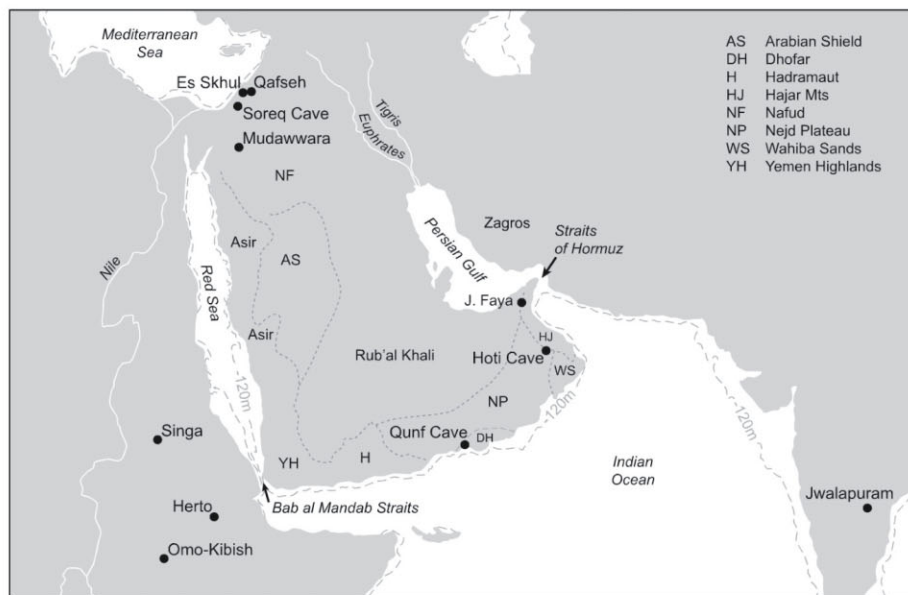


Fig. 1. The location of Jebel Faya, United Arab Emirates, along with key sites mentioned in the text. The dashed line represents the ~120-m paleoshoreline, indicating the maximum exposure of land during marine lowstands.

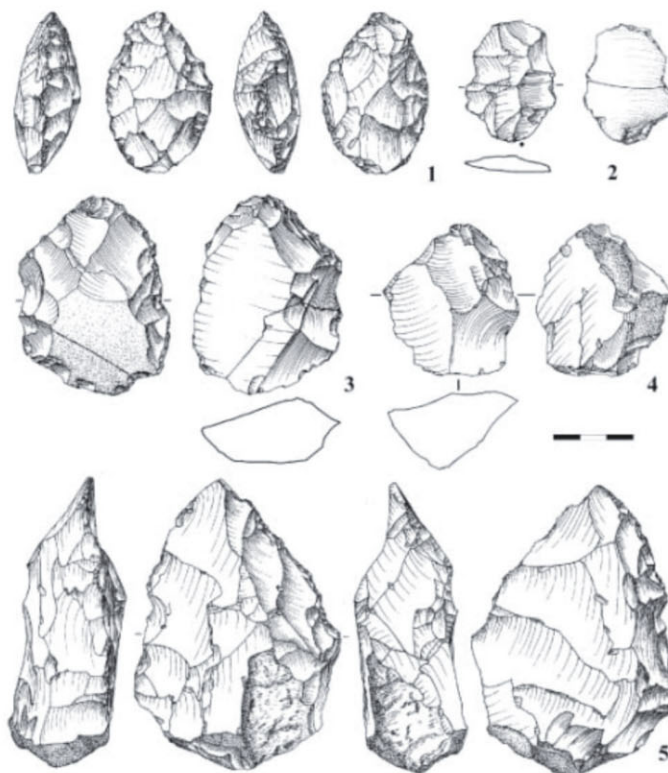


Fig. 2. Assemblage C: 1, bifacial foliate; 2, Levallois flake; 3, bifacial preform; 4, radial core; and 5, hand ax preform.

different is the reduction by *façonnage* for the production of small hand axes and foliate tool forms. Technological patterns at FAY-NE1 show greater similarities with East and northeast Africa (18) than with other sites known in Arabia. On the basis of these affinities and the contemporaneous presence of AMH in East and northeast Africa, we suggest that assemblage C occupation is attributed to AMH expanding out of Africa during early MIS 5. An autochthonous development for assemblages A and B is suggested be-

cause they bear no affinities with Middle Stone Age/Late Stone Age assemblages known from East Africa, the Upper Palaeolithic from the Levant, or the Zagros. Assemblage A occupation may have been terminated by the onset of hyperarid conditions, as indicated by the sterile sand layer. Certainly, there is no evidence for human presence at Jebel Faya between 38 and 10 ka. This hiatus coincides with a period of hyperaridity and the emplacement of major aeolian dune networks across the Arabian Peninsula (19).

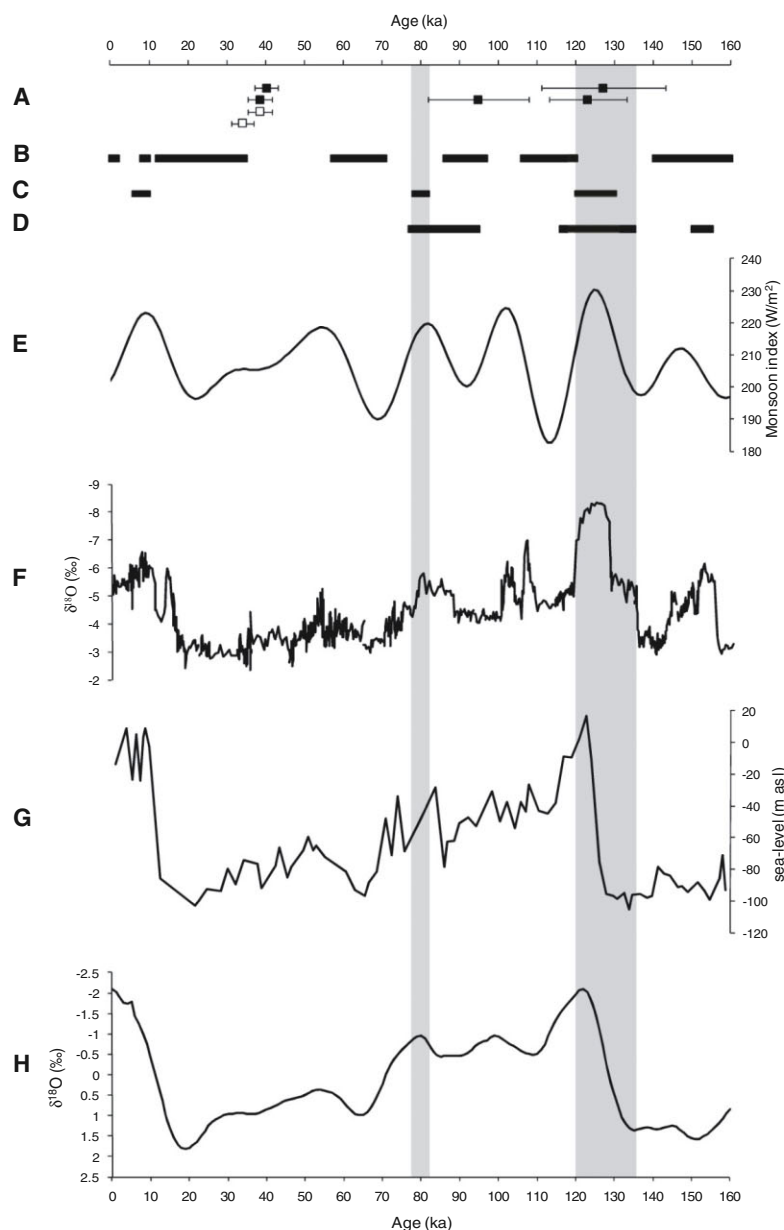


Fig. 3. Compilation of the Jebel Faya chronology with regional and global proxy records. Vertical gray bars represent periods of humidity (19). (A) OSL ages from Jebel Faya. Solid squares represent ages from within occupation phases C and A, whereas open squares represent ages from the sterile sand layer superimposed on top of assemblage A (this study). Error bars indicate 1 SE. (B) Phases of aeolian dune accumulation in the Wahiba Sands, Oman (28). (C) Timing of wet phases in Oman from Hoti and Qunf Cave speleothem records (29, 30). (D) Phases of lacustrine development, Mudawwara, Jordan (31). (E) Indian Ocean Monsoon Index (32). (F) Oxygen isotope ($\delta^{18}\text{O}$) record from Soreq Cave (33). ‰ indicates per mil. (G) Red Sea eustatic changes (20). (H) SPECMAP stacked oxygen isotope record (34).

Within the Arabian section of the southern route, the Nejd Plateau, along with the Straits of Bab al-Mandab and Hormuz, are the three major obstacles to human expansions. The Nejd Plateau is flat and today has poor vegetation cover and lacks surface water. Contrasting climatic conditions are required to allow human migration through these bottlenecks. The straits were easiest to cross during periods of low sea level, associated with glacial epochs, during which the interior of Arabia and the Nejd Plateau was hyperarid. Conversely, during interglacials, when sea level was high, the Nejd Plateau had increased vegetation density and water was more available. Consequently, the phasing of changes in eustatic sea level and terrestrial moisture availability is the key to understanding the timing of human expansion along the southern route.

We suggest that the initial expansion of AMH from East Africa to southern Arabia occurred at the transition from MIS 6 to MIS 5e, when the width of the Bab al-Mandab was at a minimum (20, 21). Once humans had crossed into southern Arabia, this migrant population would have experienced decreased predation and competition for resources. Southern Arabia may have become a secondary center for human population growth. During pluvial episodes, southern Arabia would thus have facilitated further hunter-gatherer range expansions onto the peninsula. There is evidence for wetter phases in southern Arabia at 135 to 120 ka (MIS 6-5e) and 82 to 78 ka (MIS 5a) (19) (Fig. 3). It is likely that populations expanded and moved through the interior of Arabia, as well as via the coastline, and used adaptive strategies incorporating terrestrial resources. The presence of humans at Jebel Faya early in MIS 5 indicates that a significant range expansion occurred during MIS 5e (22). The African affinity of assemblage C implies that a wetter southern Arabian climate rather than technological innovation was responsible for this range expansion (9). With more arid conditions during MIS 5d and 5b, this population probably became disconnected from that in south Arabia, as the corridor across the Nejd Plateau was lost. The continued deposition of Paleolithic artifacts at FAY-NE1, which have no known affinities to other lithic industries in the surrounding areas (16), suggests that AMH presence continued in southeast Arabia throughout MIS 5.

Because the Persian Gulf averages just 40 m in depth (23), lowered eustatic sea levels during MIS 5d and 5b, and between 75 ka and 14 ka, would have provided a land connection between southeastern Arabia and Iran (24). The former Tigris-Euphrates river system flowed through the exposed Persian Gulf and into the Indian Ocean. This landscape periodically formed a corridor for animals including Mesopotamian fallow deer, aurox, and water buffalo (25), as well as Indian elephants, which existed in northwestern Mesopotamia until early historic times (26).

Consequently, the extended Persian Gulf region is likely to have formed another population center from which early modern humans could

radiate during favorable times (22). This probably includes the reoccupation of FAY-NE1 during MIS 3 by the population that produced assemblage A. Access from southeast Arabia to the Persian Gulf and vice versa is likely to have been via the numerous wadi channels that extend from the Hajar Mountains and into the Persian Gulf basin, passing Jebel Faya to the north and the south. These channels would also have facilitated human migration by providing access to fresh water along the shores of the proto-Gulf (22, 27).

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Supporting Online Material

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Phosphorylation of ULK1 (hATG1) by AMP-Activated Protein Kinase Connects Energy Sensing to Mitophagy

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Adenosine monophosphate-activated protein kinase (AMPK) is a conserved sensor of intracellular energy activated in response to low nutrient availability and environmental stress. In a screen for conserved substrates of AMPK, we identified ULK1 and ULK2, mammalian orthologs of the yeast protein kinase Atg1, which is required for autophagy. Genetic analysis of AMPK or ULK1 in mammalian liver and *Caenorhabditis elegans* revealed a requirement for these kinases in autophagy. In mammals, loss of AMPK or ULK1 resulted in aberrant accumulation of the autophagy adaptor p62 and defective mitophagy. Reconstitution of ULK1-deficient cells with a mutant ULK1 that cannot be phosphorylated by AMPK revealed that such phosphorylation is required for mitochondrial homeostasis and cell survival during starvation. These findings uncover a conserved biochemical mechanism coupling nutrient status with autophagy and cell survival.

A highly conserved sensor of cellular nutrient status found in all eukaryotes is the adenosine monophosphate (AMP)-activated protein kinase (AMPK). In response to decreases in intracellular ATP, AMPK is activated and serves as a metabolic checkpoint, restoring ATP levels through acute regulation of metabolic enzymes and inhibition of pro-growth anabolic pathways (1). Inactivation of LKB1, the upstream kinase necessary for activation of AMPK under low-energy conditions, is a frequent event in sev-

eral forms of human cancer (2). In addition, LKB1 signaling is required in the liver for the therapeutic effect of metformin, the most prevalent type 2 diabetes drug worldwide, and LKB1 inactivation in mouse liver results in a type 2 diabetes-like metabolic disease (3). Thus the LKB1-AMPK pathway provides a direct link between tumor suppression and control of cellular and organismal metabolism.

Similar to AMPK activation, the cellular process of autophagy is initiated under nutrient-poor

and low-energy conditions as a survival mechanism to ensure availability of critical metabolic intermediates and to eliminate damaged organelles, including mitochondria (4). Autophagy is thought to be initiated under nutrient-limited conditions by a conserved kinase complex containing the serine-threonine kinase Atg1 and its associated subunits, Atg13 and Atg17 (5). In mammals, this complex is encoded by two Atg1 homologs, ULK1 and ULK2, and the subunits Atg13 and FIP200, which signal to downstream autophagy regulators through still poorly understood mechanisms. In yeast and mammalian cells, Atg1 or ULK1 activity is suppressed under nutrient-rich conditions by the TOR (target of rapamycin) complex 1 (TORC1) (6). However, biochemical events that activate Atg1 or ULK1 have not yet been identified.

We used a two-part screen to identify substrates of AMPK that mediate its effects on cell

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growth and metabolism (7). First, we used an optimal AMPK substrate motif (8) to search eukaryotic databases for proteins containing conserved candidate target sites. Many *in vivo* substrates of AMPK not only conform to this motif but also bind to the phospho-binding protein 14-3-3 inducibly upon phosphorylation by AMPK. We therefore screened for proteins that bound to recombinant 14-3-3 in wild-type (WT) but not AMPK-deficient cells, and only under conditions of energy stress, when AMPK would be active. One protein we identified that contained multiple conserved candidate AMPK phosphorylation sites and associated with 14-3-3 in an AMPK-dependent manner was the mammalian Atg1 homolog ULK1 (Fig. 1, A and B). ULK1 contains four sites (Ser⁴⁶⁷,

Ser⁵⁵⁵, Thr⁵⁷⁴, and Ser⁶³⁷) matching the optimal AMPK substrate motif, all of which are conserved in higher eukaryotes. Two of the sites are conserved back to *Caenorhabditis elegans* (Ser⁵⁵⁵ and Ser⁵⁷⁴) and in the mammalian family member ULK2, though not the more distant family members ULK3 and ULK4, which unlike ULK1 and ULK2 are not thought to function in autophagy. Indeed, endogenous AMPK subunits co-immunoprecipitated with ULK1 and ULK2 but not ULK3 (fig. S1), and AMPK subunits were found in unbiased identifications of proteins co-immunoprecipitating with overexpressed ULK2 (fig. S2), which is consistent with recent proteomic analyses (9). To examine ULK1 *in vivo* phosphorylation sites, we used tandem mass spec-

trometry on epitope-tagged ULK1 isolated from cells treated with or without the mitochondrial complex I inhibitor phenformin (10). We detected peptides spanning three of the four candidate AMPK sites in ULK1 (Ser⁵⁵⁵, Thr⁵⁷⁴, and Ser⁶³⁷), and all three were phosphorylated only after phenformin treatment (figs. S3 and S4). To examine whether ULK1 could serve as a direct substrate for AMPK *in vitro*, we created a kinase-inactive allele (K46I) (11) to remove its autophosphorylation. AMPK phosphorylated ULK1 to a greater extent than it did an established substrate, Raptor (Fig. 1C and fig. S5), which may reflect the presence of at least four potential AMPK sites in ULK1, as compared with Raptor, which has two reported AMPK sites (8). We gen-

Fig. 1. ULK1 is a conserved substrate of AMPK.

(A) Clustal alignment of four conserved sites in ULK1 and two sites in ULK2 matching the optimal AMPK substrate motif. (B) ULK1 and glutathione S-transferase (GST) or GST-14-3-3 expression vectors were transfected into human embryonic kidney (HEK) 293T cells, and placed in media containing 20 μ M STO-609 (STO), vehicle (veh), or 5 mM phenformin (Phen) for 1 hour. Cell lysates and GST pull-downs were immunoblotted as indicated. (C) *In vitro* kinase assays with myc-tagged catalytically inactive (KI: K46I) ULK1 or myc-tagged WT raptor that were immunoprecipitated from HEK293T cells and used as substrates for purified active AMPK in the presence of ³²P-(C)-ATP. (D) HEK293T cells transfected with myc-tagged WT ULK1 or indicated serine-to-alanine ULK1 mutants were treated with either vehicle or 1 mM phenformin for 1 hour or were cotransfected with a constitutively active AMPK α 1 (aa1-312) mammalian expression vector (12). Proteins from lysates were immunoblotted with phospho-specific antibodies as indicated. (E) *In vitro* kinase assays using myc-ULK1 and purified AMPK as above. Phosphorylation of myc-ULK1 detected through immunoblotting with indicated phospho-specific antibodies. (F) Primary MEFs were treated with 2 mM AICAR or vehicle for 1 hour. Lysates were immunoblotted as indicated, including detection of endogenous ULK1 P-Ser⁵⁵⁵.

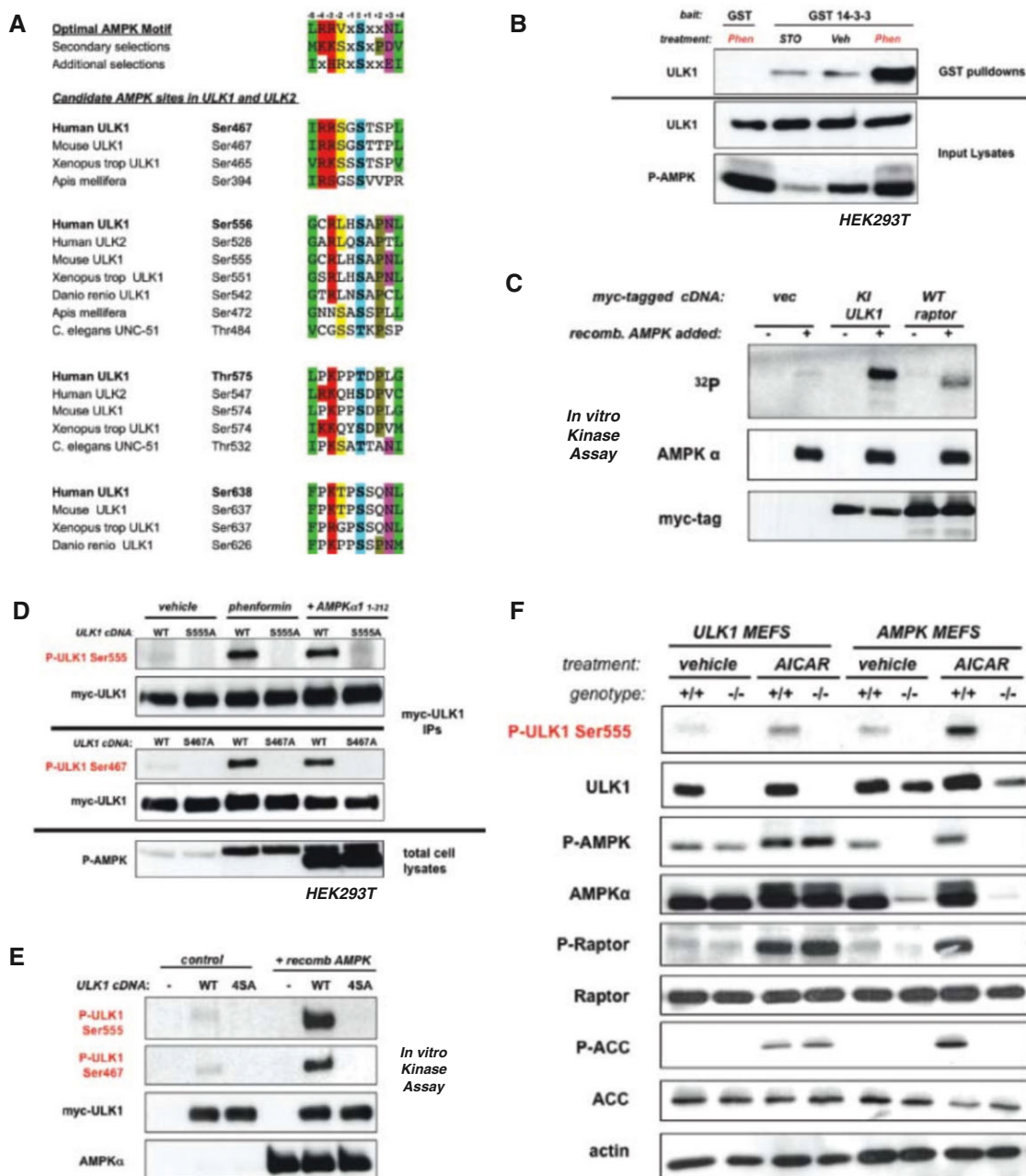


Fig. 2. Genetic deficiency for AMPK or ULK1 in mouse liver or primary mouse hepatocytes results in autophagy defects. **(A)** Liver lysates from littermate-matched mice were immunoblotted for the indicated antibodies. p62-to-actin ratio calculated from densitometry performed on immunoblots. Data shown as mean $\pm$ SEM. $*P < 0.01$. **(B)** Primary hepatocytes derived from ULK1^{+/+} or ULK1^{-/-} mice or AMPK α 1^{+/+} α 2^{+/+} or AMPK α 1^{-/-} α 2^{L/L} as described in (7) were placed in media containing 2 mM metformin (met) or vehicle (veh) for 2 hours. Lysates were immunoblotted with the indicated antibodies. **(C)** TEM was performed on primary mouse hepatocytes of the indicated genotypes, revealing accumulation of mitochondria in both AMPK- and ULK1-deficient cells. Red, mitochondria; blue, cytoplasm; green, nuclei; yellow, lipid droplets. **(D)** Primary mouse hepatocytes of the indicated genotypes stained by means of immunocytochemistry for the mitochondrial marker TOM20 (red) and nuclei (blue). Scale bar, 10 μ m.

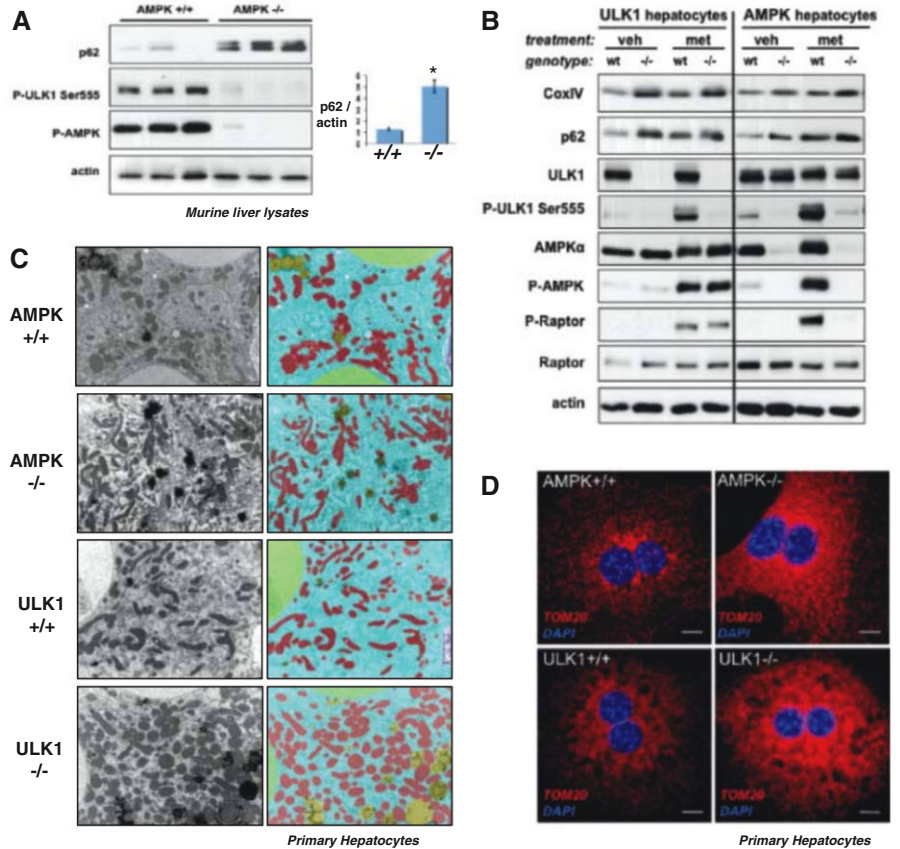
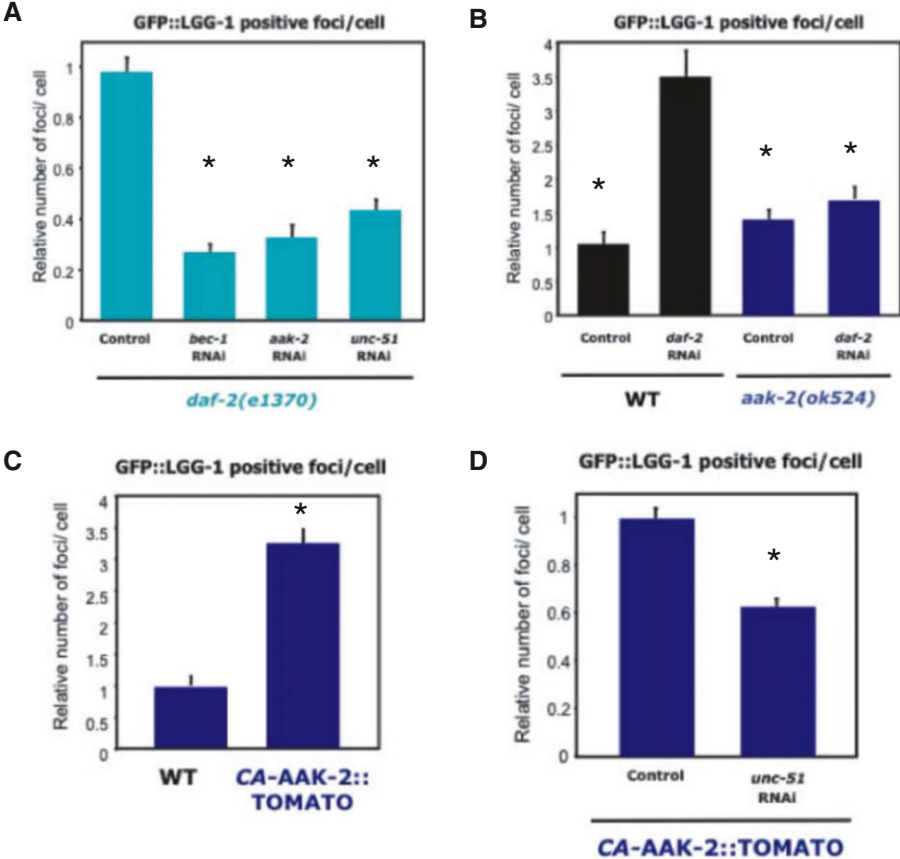


Fig. 3. AMPK is necessary and sufficient for autophagy induction in *C. elegans*. **(A)** Insulin receptor *daf-2(e1370)* mutant worms expressing GFP::LGG-1 (equivalent to GFP-LC3) were treated with control RNAi or RNAi against *bec-1* (Beclin), *aak-2* (AMPK α 2), or *unc-51* (ULK1), and the number of LGG-1/LC3-positive puncta per hypodermal seam cell were quantified. **(B)** *aak-2(ok524)* mutants or WT N2 (WT) animals expressing GFP::LGG-1 were treated with control or *daf-2* RNAi and scored for LGG-1 positive puncta per seam cell. **(C)** Transgenic worms expressing constitutively active AAK-2 (12) (amino acids 1-321)::TOMATO (CA-AAK-2::TOMATO) fusion or controls (WT) were analyzed for LGG-1-positive puncta per seam cell. **(D)** Animals expressing both CA-AAK-2(1-321)::TOMATO and GFP::LGG-1 were treated with control or *unc-51* RNAi and scored for LGG-1/LC3-positive puncta per seam cell. All panels show relative counts; see fig. S10 for details. Data shown as mean $\pm$ SEM. $*P < 0.0001$.

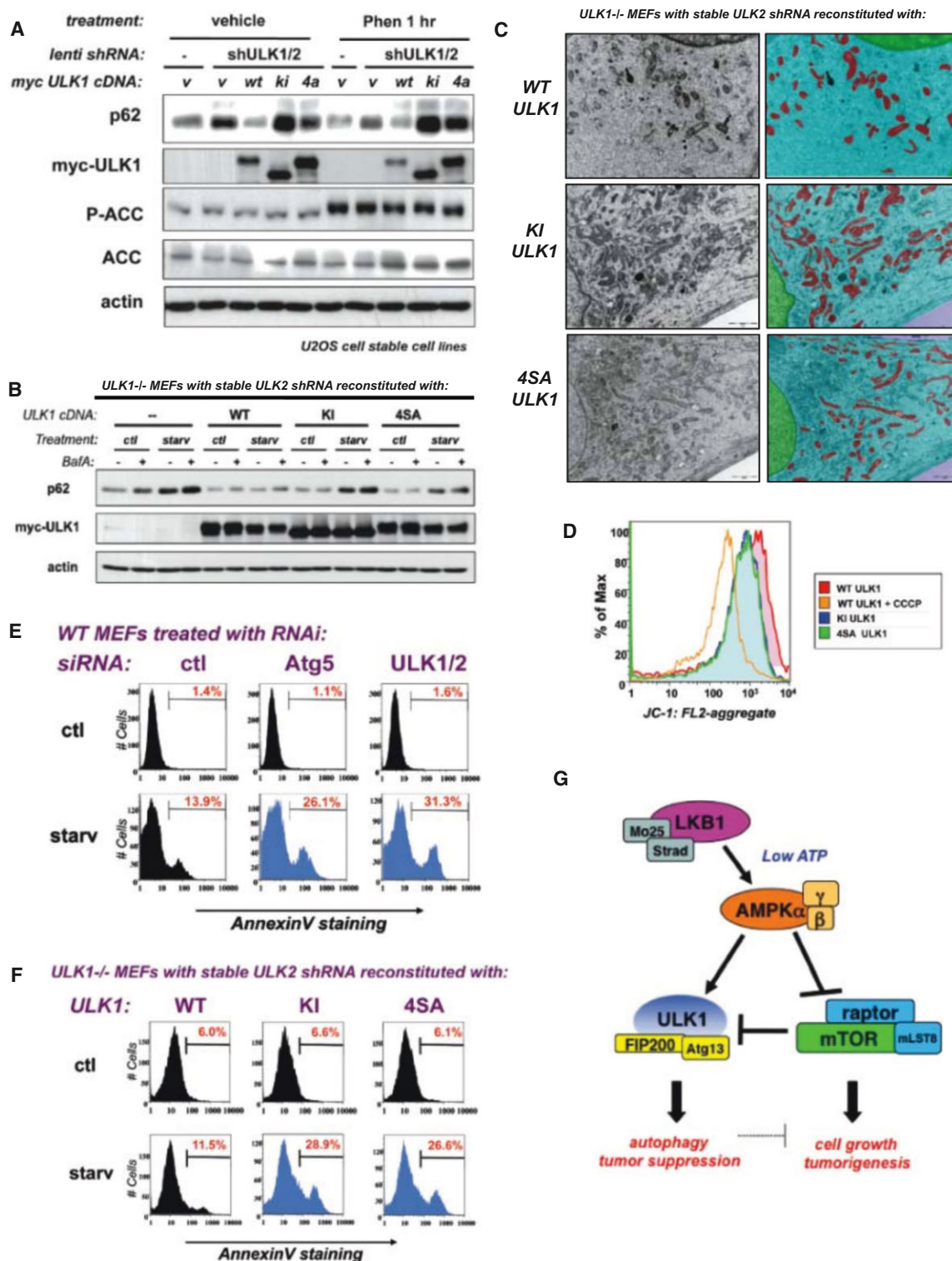


erated phosphospecific antibodies against Ser⁴⁶⁷ and Ser⁵⁵⁵ of ULK1. Phosphorylation of both sites was induced by means of phenformin treatment or expression of ULK1 with a constitutively active AMPK α 1 allele (12) in the absence of

energy stress (Fig. 1D). Purified AMPK also induced phosphorylation at these sites in an *in vitro* kinase assay, which is consistent with their direct phosphorylation (Fig. 1E). Using AMPK- and ULK1-deficient primary mouse embryonic fibro-

blasts (MEFs) or matched control WT MEFs, we observed phosphorylation of endogenous ULK1 on Ser⁵⁵⁵ in an AMPK-dependent manner after treatment of cells with the AMP-mimetic aminoimidazole carboxamide ribonucleotide (AICAR)

Fig. 4. AMPK phosphorylation of ULK1 is required for mitophagy and cell survival upon nutrient deprivation. (A) U2OS cells stably expressing mouse WT, KI, or AMPK 4SA ULK1 cDNA or the empty retroviral vector (v) along with a shRNA against endogenous human ULK1 and ULK2 were placed in media containing 5 mM phenformin (Phen) or vehicle for 1 hour. Lysates were immunoblotted as indicated. (B) ULK1^{-/-} MEFs stably expressing WT, KI, or 4SA ULK1 cDNA or the empty retroviral vector (v) along with a shRNA against endogenous ULK2 were placed in Earle's balanced salts solution starvation media (starv) or control media (ctl) for 6 hours in the presence or absence of BafilomycinA (BafA) and immunoblotted as indicated. (C) Cells from (B) analyzed with TEM and Inform morphometric software. Red, mitochondria; blue, cytoplasm; green, nuclei. (D) Fluorescence-activated cell sorting (FACS) analysis on cells from (B), which were stained with JC-1 under basal conditions or with the mitochondrial uncoupler carbonyl cyanide m-chloro phenyl hydrazine (CCCP) as a control in order to measure mitochondrial membrane potential. Compromised mitochondrial membrane potential is shifted to the left, as observed in cells treated with CCCP. (E) WT MEFs transfected with 20 nM siRNA pools to a universal control (ctl), mouse Atg5, or mouse ULK1 and ULK2 for 72 hours were then placed in starvation medium (starv) or standard media (ctl) for 12 hours, and cell death was scored by means of AnnexinV-FACS. (F) Cells from (B) were placed in starvation medium (starv) or standard media (ctl) for 12 hours, and cell death was scored by means of AnnexinV-FACS. (G) Model for AMPK activation of ULK1 in a two-pronged mechanism via direct phosphorylation of ULK1 and inhibition of mTORC1 suppression of ULK1.



(Fig. 1F). The phosphorylation of ULK1 in these cells paralleled that of two bona fide AMPK substrates, acetyl-CoA carboxylase (ACC) and Raptor (Fig. 1F and fig. S6).

We examined the phenotypic consequences of AMPK or ULK1 deficiency on markers of autophagy in mouse liver and primary hepatocytes. Immunoblot and immunohistochemical analysis of AMPK-deficient livers (13) showed accumulation of the p62 protein (Fig. 2A and fig. S7), whose selective degradation by autophagy has established it as a widely used marker of this process (14). p62 contains a ubiquitin-associated (UBA) ubiquitin-binding domain, which mediates binding to ubiquitinated cargo targeted for autophagy-mediated degradation (14). Consistent with this function, p62 aggregates colocalized with ubiquitin aggregates in AMPK-deficient livers (fig. S7). p62 is recruited to mitochondria targeted for mitophagy and is involved in mitochondrial aggregation and clearance (15, 16). ULK1-deficient mice exhibit accumulation of defective mitochondria in mature red blood cells, which are normally devoid of mitochondria (17). Given the aberrant accumulation of p62 in the absence of AMPK in mouse liver and the fact that rodent hepatocytes undergo substantial mitophagy upon culturing (18), we examined whether AMPK or ULK1 deficiency in primary hepatocytes might exhibit mitochondrial defects. Protein levels of p62 and the mitochondrial marker protein CoxIV were similarly elevated in lysates from AMPK- or ULK1-deficient hepatocytes cells but not from WT controls (Fig. 2B and fig. S8). Increased phosphorylation of endogenous ULK1 Ser⁵⁵⁵ was observed in WT but not AMPK-deficient hepatocytes after AMPK activation by metformin treatment (Fig. 2B). Further analysis of the ULK1 and AMPK hepatocytes by use of transmission electron microscopy (TEM) revealed elevated levels of abnormal mitochondria, which was analyzed quantitatively with morphometric software (Fig. 2C, right). Similar to findings in other autophagy-mutant hepatocytes (19), the number of mitochondria per cell was significantly increased in AMPK- and ULK1-deficient hepatocytes as compared with that of WT controls (fig. S9), which is also seen with immunocytochemical staining for the mitochondrial membrane protein TOM20 (Fig. 2D).

Given the conservation of AMPK sites in ULK1, we examined whether these two proteins function together to play conserved roles in autophagy in the nematode *C. elegans*. In a reporter assay based on the *C. elegans* LC3 homolog LGG-1 (20, 21), we observed that loss of insulin signaling through genetic mutation [*daf-2* (*el370*)] or RNA interference (RNAi) against the insulin receptor *daf-2* resulted in increased numbers of green fluorescent protein (GFP)::LGG-1-positive foci in hypodermal seam cells, which is indicative of increased autophagy and consistent with the established role for insulin signaling in the suppression of autophagy in *C. elegans* (20–24). *daf-2* mutant worms treated with RNAi to *aak-2* or *unc-51*, the AMPK and

ULK1 orthologs, respectively, resulted in a decrease in abundance of LGG-1-containing puncta (Fig. 3A). *daf-2* RNAi failed to increase the number of LGG-1-positive foci in AMPK-deficient worms (Fig. 3B). These data indicate that both AMPK and ULK1 have critical roles in autophagy induced by means of reduced insulin signaling in *C. elegans*. Transgenic worms expressing constitutively active AMPK exhibited an approximately threefold increase in the number of LGG-1-positive foci in seam cells as compared with the number of foci in controls (Fig. 3C). The number of LGG-1-positive foci was significantly reduced when these animals were fed *unc-51* RNAi (Fig. 3D) (all data can be found in fig. S10). These observations indicate that AMPK activation is sufficient to induce autophagy in worms, and ULK1 is required for this induction.

To test whether AMPK phosphorylation of ULK1 is required for ULK1 function, we stably introduced WT, catalytically inactive (KI), or the AMPK nonphosphorylatable (4SA) ULK1 cDNA into human osteosarcoma U2OS cells in which we subsequently reduced endogenous ULK1 and ULK2 with lentiviral short hairpin RNAs (shRNAs) against each (fig. S11). U2OS cells stably expressing ULK1 and ULK2 shRNA exhibited increased amounts of p62 indicative of defective autophagy as compared with that of parental U2OS cells infected with an empty lentiviral vector (Fig. 4A; compare lane 1 and 2). Stable retroviral reconstitution of a myc-tagged WT ULK1 cDNA, but not the 4SA or KI mutant, restored p62 degradation (Fig. 4A, lanes 3 to 5, and fig. S12). Furthermore, we reconstituted ULK1^{−/−} MEFs that were also knocked down for endogenous ULK2 (fig. S13) with WT, KI, or 4SA ULK1 cDNAs and examined the extent of autophagy after placement of these cell lines into starvation media. MEFs deficient for ULK1 and ULK2 contained elevated levels of p62 upon starvation. Cells reconstituted with WT ULK1 had reduced p62 levels, unlike the KI- or 4SA-expressing cells, which behaved like the ULK-deficient state (Fig. 4B and fig. S14). To test whether the 4SA mutant exhibited effects on mitochondrial homeostasis, we used TEM and mitochondrial-selective dyes on the WT, KI, and 4SA ULK1 stably reconstituted ULK-deficient MEFs. TEM and Mitotracker Red staining revealed that the KI- and 4SA-ULK1-expressing cells had altered mitochondrial homeostasis as compared with that of WT ULK1 cells, denoted by increases in the overall number and aberrant morphology of mitochondria (Fig. 4C and figs. S18 and S19). The altered cristae and aberrant morphology of the mitochondria in the KI- and 4SA-ULK1-reconstituted cells was enhanced upon starvation (fig. S19). To test whether these mitochondria were functionally impaired, we analyzed the mitochondrial membrane potential with the activity-dependent JC-1 dye, which revealed defects in KI- and 4SA-reconstituted MEFs (Fig. 4D).

A hallmark of cells defective for autophagy is a predisposition to undergo apoptosis after stress

stimuli that normally would activate autophagy to promote cell survival (25). We examined how ULK1/2 deficiency would compare with loss of central downstream autophagic regulator such as Atg5 in terms of requirement for cell survival after starvation. WT MEFs were treated with control, Atg5, or combined ULK1 and ULK2 small interfering RNA (siRNA) and analyzed for effects on cell viability after being placed into starvation conditions. Simultaneous depletion of ULK1 and ULK2 mirrored the magnitude and kinetics of cell death observed with Atg5 loss upon starvation (Fig. 4E and fig. S20). We next investigated whether mutation of the AMPK sites in ULK1 might also mimic ULK1/2 loss of function in this cell survival assay. ULK-deficient MEFs reconstituted with WT, but not KI or 4SA ULK1, restored cell survival upon starvation (Fig. 4F). ULK1-deficient cells expressing the KI or 4SA mutant ULK1 showed rates of cell death similar to those of WT MEFs treated with ULK1 and ULK2 siRNA. Thus, loss of the AMPK sites in ULK1 mimics complete loss of ULK1 and ULK2 in control of cell survival after nutrient deprivation.

Our findings reveal a direct connection between energy sensing and core conserved autophagy proteins. In mammals, phosphorylation of ULK1 by AMPK is required for ULK1 function in the response to nutrient deprivation. Because AMPK suppresses mammalian TOR (mTOR) activity and mTOR inhibits ULK1 (26–30), AMPK controls ULK1 via a two-pronged mechanism, ensuring activation only under the appropriate cellular conditions (Fig. 4G and fig. S21). There are a number of physiological and pathological contexts in which this pathway is likely to play a critical role (31). Beyond the conserved nature of these signaling events and the role of some autophagy genes as tumor suppressors (25, 32), AMPK is defective in a variety of human cancers bearing inactivating mutations in its upstream kinase LKB1. Thus, ULK1 may have a central role in the beneficial effects of the LKB1/AMPK pathway on tumor suppression or in treatment of metabolic disease, as observed here with metformin stimulation of ULK1 phosphorylation in liver and the profound defect in autophagy in AMPK-deficient livers. ULK1-dependent effects on mitochondrial homeostasis and cell survival may represent additional beneficial effects of metformin and other AMPK activators in overall organismal health and life span (33).

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Effects of Experimental Seaweed Deposition on Lizard and Ant Predation in an Island Food Web

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The effect of environmental change on ecosystems is mediated by species interactions. Environmental change may remove or add species and shift life-history events, altering which species interact at a given time. However, environmental change may also reconfigure multispecies interactions when both species composition and phenology remain intact. In a Caribbean island system, a major manifestation of environmental change is seaweed deposition, which has been linked to eutrophication, overfishing, and hurricanes. Here, we show in a whole-island field experiment that without seaweed two predators—lizards and ants—had a substantially greater-than-additive effect on herbivory. When seaweed was added to mimic deposition by hurricanes, no interactive predator effect occurred. Thus environmental change can substantially restructure food-web interactions, complicating efforts to predict anthropogenic changes in ecosystem processes.

Global environmental change is expected to have a profound impact on the structure and function of ecological communities by changing interactions between their component species. Range shifts, extinctions, and species introductions change community composition, deleting some interactions and adding others (1, 2). In addition, changes in the seasonal timing of migration and other life-history events can produce phenological mismatches, which can affect communities even when species composition is unchanged (1–3). The absence of alterations in species composition and phenology, however, does not necessarily mean that ecosystems will remain unaltered (4): Environmental change can also influence ecosystem processes by reconfiguring interactions in communities whose species lists remain intact (5–9).

An important aspect of global environmental change is the mobilization and transport of resources between ecosystems. Seaweed deposition in particular is likely to become a more common feature in shoreline ecosystems as anthropogenic effects (such as overfishing and eutrophication) facilitate a shift toward algae-dominated marine ecosystems (10). Furthermore, intense storms, which are associated with the deposition of large amounts of seaweed (11, 12), have increased in frequency—a trend that is expected to continue with increasing global warming (13). Such pulsed inputs of external resources can increase prey availability, “subsidizing” in situ predators (14–17) and altering predator effects on lower trophic levels (12, 16, 18–21).

We used a whole-island field experiment in the Bahamas to probe how major seasonal pulses of seaweed deposition—mimicking what occurs in an active storm year (22)—influence the effects of multiple predators on herbivory in a terrestrial food web. Twelve small islands (one is shown in Fig. 1A), six with lizards and six naturally lizard-free, were used in the experiment. Seaweed was added or removed from islands in a

crossed design; each combination of seaweed and lizard presence or absence was represented by three islands. Seaweed was manipulated in October and December of 2008, coinciding with that season when large storms are most likely to cause natural deposition events. On seaweed-addition islands, 0.4 to 1.4 kg/m² of seaweed was distributed patchily throughout each island, mimicking what occurs after a large storm (22). The removal treatment maintained seaweed at a level near zero, which is consistent with natural levels of about half of the islands before manipulation. Ant exclusions were established on branches of four (three in one case) *Conocarpus erectus* plants on each island; ants were excluded with a sticky resin, which lizards were able to bypass by crossing a narrow gap between wire mesh cones (Fig. 1B). Each of the four predator treatments in the experiment—(i) ants and lizards absent; (ii) ants present and lizards absent; (iii) ants absent and lizards present; and (iv) ants and lizards present—was represented by 12 branches in the absence of seaweed subsidy and 11 or 12 branches in the presence of seaweed subsidy.

Anolis sagrei was the only lizard species on the lizards-present islands. Previous experiments in this ecosystem demonstrated that *A. sagrei* reduced herbivory on *C. erectus* (23, 24), one of the most common plants in shoreline habitats. Extrafloral nectaries on *C. erectus* foliage attract mutualistic ants, which can also decrease herbivory (25). The possibility that these two predators interactively affect lower trophic levels (26) had not been explored in previous studies.

Figure 1C shows a working model of the main food web components in our system. Seaweed deposits support an abundance of detritivores, which attract both ants and lizards (12). The most common herbivorous arthropods include Coleoptera, Lepidoptera, and Hemiptera (12). Previous studies in this system indicate that a shift in predator foraging behavior after two closely spaced pulses of seaweed is associated with increased herbivory on *C. erectus* (12), suggesting that the subsidies

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interfere with herbivore suppression by predators (27).

The present study shows that seaweed subsidies diminish the effect of predators on *C. erectus* by eliminating the greater-than-additive effect of

ants and lizards on herbivory found in the absence of seaweed subsidies. Specifically, without seaweed the combined effect of ants and lizards on herbivory was more than three times greater than expected on the basis of the sum of their individual

effects (Fig. 2, left). In other words, the difference between the treatment with both predators present and the no-predators treatment was three times greater than the difference between the ants/no-lizards treatment and the no-predators treatment plus the difference between the lizards/no-ants treatment and the no-predators treatment. In contrast, in the presence of seaweed subsidies there was no interactive effect of ants and lizards on herbivory (Fig. 2, right). Ant abundance increased on islands with seaweed subsidies—the (least-squares) mean ant abundance on seaweed-addition islands was higher than that on seaweed-removal islands—but was not affected by lizards (Fig. 3A). In contrast to ants, lizards did not increase in overall abundance on subsidized islands (Fig. 3B).

We suggest that the synergistic effect of lizards and ants on herbivory in the absence of seaweed subsidies derives from the fact that these two predators are active at different times of day. The only lizard species present, *A. sagrei*, is diurnal. In contrast, the dominant ant species (comprising 88% of all pan-trapped ants), *Camponotus tortuganus*, is nocturnal. This temporal partitioning of foraging activity may create a dilemma for herbivores: They can avoid *A. sagrei* by feeding at night, and they can avoid *C. tortuganus* by feeding during the day, but they cannot simultaneously avoid both types of predators. This hypothesis is analo-

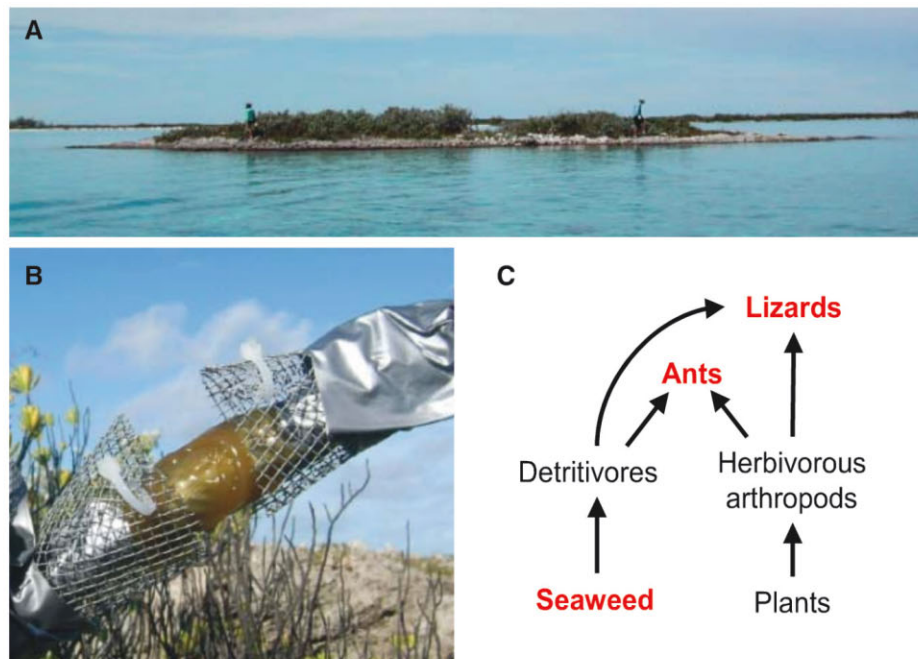


Fig. 1. Study system. (A) One of the twelve experimental islands. (B) An ant-exclusion treatment on one of the experimental plants. The wire mesh cones on either side allow lizards to pass over the sticky resin barrier. (C) Working hypothesis of food-web relationships on the small islands used in this experiment. Red text denotes the components that were manipulated in this study.

Fig. 2. Effects of seaweed, lizards, and ants on herbivory in *C. erectus*. (A) Leaf damage on *C. erectus* in four predator treatments on seaweed-removal and seaweed-addition islands. Data are mean $\pm$ SE; $n = 11$ branches for the seaweed addition–lizards treatments and 12 branches for all other treatments. The interactive effect of lizards and ants on *C. erectus* leaf damage differed between seaweed treatments [mixed model analysis of covariance (ANCOVA); seaweed* lizard*ant interaction: $F_{1,39} = 6.0$, $P = 0.02$]. On seaweed-removal islands, there was a greater-than-additive effect of ants and lizards on herbivory (mixed model ANCOVA; lizard*ant interaction on seaweed-removal islands: $F_{1,20} = 11.5$, $P = 0.003$), but there was no such interactive effect on seaweed-addition islands (mixed model ANCOVA; lizard*ant interaction on seaweed-addition islands: $F_{1,19} = 0.16$, $P = 0.7$). (B) Predator effects on leaf damage. Effect sizes were calculated as $\ln$ (leaf damage with predators/leaf damage without predators) by using least-squares means from the mixed model ANCOVA described in (22).

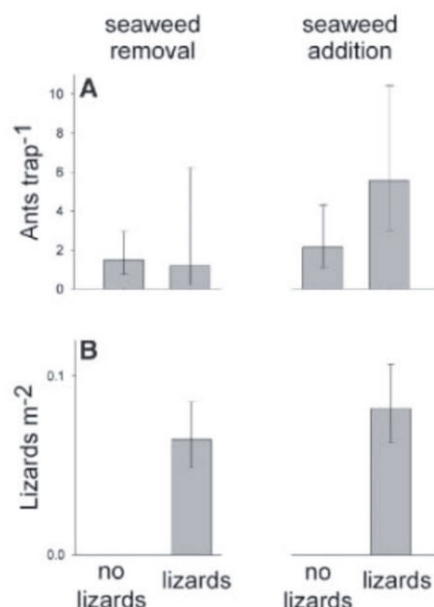
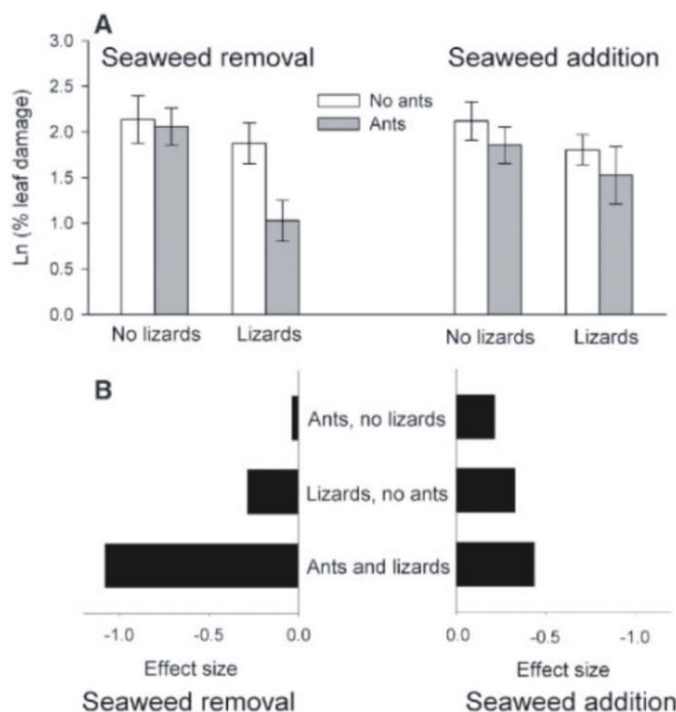


Fig. 3. Effects of seaweed on ant and lizard abundance. (A) Ant abundance in pan traps on experimental islands. Ant abundance was higher on seaweed-addition islands than seaweed-removal islands (ANCOVA; $F_{1,7} = 7.7$, $P = 0.03$); there was no difference between lizard and no-lizard islands [analysis of variance (ANOVA); $F_{1,7} = 0.15$, $P = 0.71$]. (B) Lizard abundance on experimental islands. There was no effect of seaweed on lizard density (ANOVA; $F_{1,4} = 0.38$, $P = 0.60$). Data are mean $\pm$ SE of log-transformed abundances; $n = 3$ islands for each treatment combination.

gous to what has been observed when predators exhibit spatial complementarity in foraging: Prey seeking to escape one predator enter habitats in which they are more vulnerable to the other predator (26). In addition to eliminating prey refuges, temporal partitioning may reduce the frequency of antagonistic interactions between predators, which probably explains why lizards did not affect ant abundance in our study.

When seaweed subsidies were added to the system, the greater-than-additive effect of ants and lizards on herbivory was not present. We suggest that the seaweed caused both ants and lizards to spend more time on the ground foraging for detritivores associated with seaweed deposits, reducing their combined impact on herbivory. This hypothesis is consistent with the results of an earlier study, in which seaweed was added or removed from shoreline plots on large islands (12). Despite higher abundances of both ants and lizards in seaweed-addition plots, *C. erectus* sustained higher levels of herbivory. An analysis of carbon-stable-isotope signatures indicated that lizard diets contained more marine-derived prey in seaweed-addition plots, and ants were observed foraging for detritivores in the seaweed deposits, suggesting that shifts in predator foraging behavior were responsible for the observed increases in herbivory (12). In the current experiment, the increase in ant density in pan traps may have been caused by a shift in foraging patterns, although an increase in the overall abundance of ants resulting from increased food supply may have also been contributory. Although lizard density increased in response to seaweed subsidies in mainland plots (12), we observed no such increase in the current study. We suggest that the absence of a numerical response in lizards on small islands was caused by (i) the inability of lizards to immigrate to the experimental sites from surround-

ing areas (as they could in the previous, mainland-plot experiment) and (ii) the lack of reproductive activity during much of the study period (12). Because emigration and reproductive lags influence the timing of predator responses to subsidy, we expect the long-term impact of pulsed seaweed subsidies on predator effects to depend on the frequency of pulses and degree of habitat isolation (15, 16). Seaweed did not appear to cause a reduction in the effects of either ants or lizards by themselves (Fig. 2B), suggesting that the interactive effect of these two predators is more sensitive to subsidy than their individual effects.

Predicting the effects of environmental change on ecosystems is an important challenge. There is increasing recognition that species interactions strongly influence how environmental change affects ecosystem processes, complicating efforts to make reliable forecasts (28, 29). Our results show that large seaweed-deposition events affect the structure and function of an ecological community by reconfiguring the effects of multiple predators on lower trophic levels. This suggests that predictions that are based on single-species responses or pairwise interactions may not adequately represent community responses to environmental perturbations. Experiments such as the one we report here, conducted at a spatial scale large enough to capture community-wide dynamics, are particularly relevant for conservation and management decisions in the face of ever-increasing anthropogenic disturbances.

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Supporting Online Material

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Materials and Methods

SOM Text

Figs. S1 to S3

References

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Metagenomic Discovery of Biomass-Degrading Genes and Genomes from Cow Rumen

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The paucity of enzymes that efficiently deconstruct plant polysaccharides represents a major bottleneck for industrial-scale conversion of cellulosic biomass into biofuels. Cow rumen microbes specialize in degradation of cellulosic plant material, but most members of this complex community resist cultivation. To characterize biomass-degrading genes and genomes, we sequenced and analyzed 268 gigabases of metagenomic DNA from microbes adherent to plant fiber incubated in cow rumen. From these data, we identified 27,755 putative carbohydrate-active genes and expressed 90 candidate proteins, of which 57% were enzymatically active against cellulosic substrates. We also assembled 15 uncultured microbial genomes, which were validated by complementary methods including single-cell genome sequencing. These data sets provide a substantially expanded catalog of genes and genomes participating in the deconstruction of cellulosic biomass.

Biofuels derived from lignocellulosic plant material represent an important renewable energy alternative to transportation fossil

fuels (1, 2). A major obstacle to industrial-scale production of fuel from lignocellulose lies in the inefficient deconstruction of plant material, owing

to the recalcitrant nature of the substrate toward enzymatic breakdown and the relatively low activity of currently available hydrolytic enzymes. Although the success of protein engineering to improve the performance of existing lignocellulose-degrading enzymes has been limited (3), retrieving enzymes from naturally evolved biomass-degrading microbial communities offers a promising strategy for the identification of new lignocellulolytic enzymes with potentially improved activities (4).

Metagenomics, the direct analysis of DNA from environmental samples, represents a strategy for discovering diverse enzymes encoded in nature (5, 6). Although metagenomics has been used

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successfully to identify enzymes with desired activities (7), it has relied primarily on relatively low-throughput function-based screening of environmental DNA clone libraries (8, 9). Sequence-based metagenomic discovery of complete genes from environmental samples has been limited by the microbial species complexity of most environments and the consequent rarity of full-length genes in low-coverage metagenomic assemblies (8, 10, 11).

In this study, we generated 268 gigabases of metagenomic sequence data from the microbiota in cow rumen to identify genes and genomes participating in biomass deconstruction. To isolate rumen microbes associated with defined plant substrates for subsequent genomic assessment, we incubated biomass-containing nylon bags in two fistulated cows (12) (Fig. 1 and table S1). We isolated organisms that had become adherent to the plant fiber material during incubation in an attempt to target microbes specifically involved in biomass degradation.

We used switchgrass (*Panicum virgatum*), a promising cellulosic energy crop (13), as the plant substrate for our studies. To determine the cow's ability to degrade this substrate, we compared the chemical composition of the switchgrass before and after rumen incubation. Switchgrass degradation was substantial (37% dry mass reduction after 72 hours of incubation). Further analysis confirmed that the decrease in mass of the switchgrass fiber was largely due to degradation of both cellulose and hemicellulose, which together accounted for 72% of the reduction in dry mass during incubation (table S2). The remaining reduction in dry mass is likely in large part due to the degradation of pectin, protein, and other components of plant biomass (14). These results indicate that the cow rumen microbiota is able to degrade this fiber source and support previous observations that the rumen environment contains some of the most cellulolytic mesophilic microbes described from any habitat (15).

To examine whether a unique fiber-degrading microbial community was enriched on switchgrass incubated within the nylon bags, we compared the community composition of microbes adherent to rumen-incubated switchgrass to the microbial population from bulk rumen fluid. We used pyrotag sequencing of small subunit ribosomal RNA genes (16) to identify operational taxonomic units (OTUs) in two fistulated cows for each of the two samples. Rarefaction analysis indicated that the pyrotag sequencing depth was sufficient to capture the vast majority of OTUs in each sample and suggests that about 1000 different OTUs were present in each of these samples (fig. S1), which is consistent with previous estimates of microbial complexity in the rumen (17). Comparison of the OTUs identified in the switchgrass fiber-adherent community and the community present in rumen fluid revealed overlaps between cows and substrates, as well as reproducible enrichment of specific bacterial phylotypes in the switchgrass-adherent fraction (18).

We targeted a single sample of switchgrass-adherent rumen microbes for deep metagenomic sequencing with the goal of maximizing the likelihood of obtaining large contiguous stretches of overlapping sequence reads (contigs) containing full-length lignocellulolytic genes. We generated several sequencing libraries from this sample with paired-end read separations (equivalent to insert sizes in clone-based libraries) of 200 base pairs (bp), 300 bp, 3 kbp, and 5 kbp. Massively parallel sequencing (19) from all libraries yielded 1.5 billion read pairs, ranging in length from 2×36 bp to 2×125 bp and amounting to a total of 268 Gbp of sequence information. A summary of the library and sequencing technologies used is provided in table S3.

To identify candidate carbohydrate-active genes from this metagenomic sequence data set,

we performed de novo assembly and predicted 2,547,270 open reading frames (ORFs). The average ORF length was 542 bp, and 55% of the ORFs were predicted to represent full-length genes. All predicted genes were screened for candidate proteins with potential enzymatic activity toward plant cell wall polysaccharides. To minimize the dependence on overall sequence similarity of candidate genes to known carbohydrate-active enzymes, we searched candidate genes for the presence of individual predicted functional domains, rather than global sequence similarity to known carbohydrate-active enzymes. We identified 27,755 candidate genes with a significant match to at least one relevant catalytic domain or carbohydrate-binding module (18) (table S4). The sequence domains identified in our sample were largely consistent with a prokaryotic origin of can-

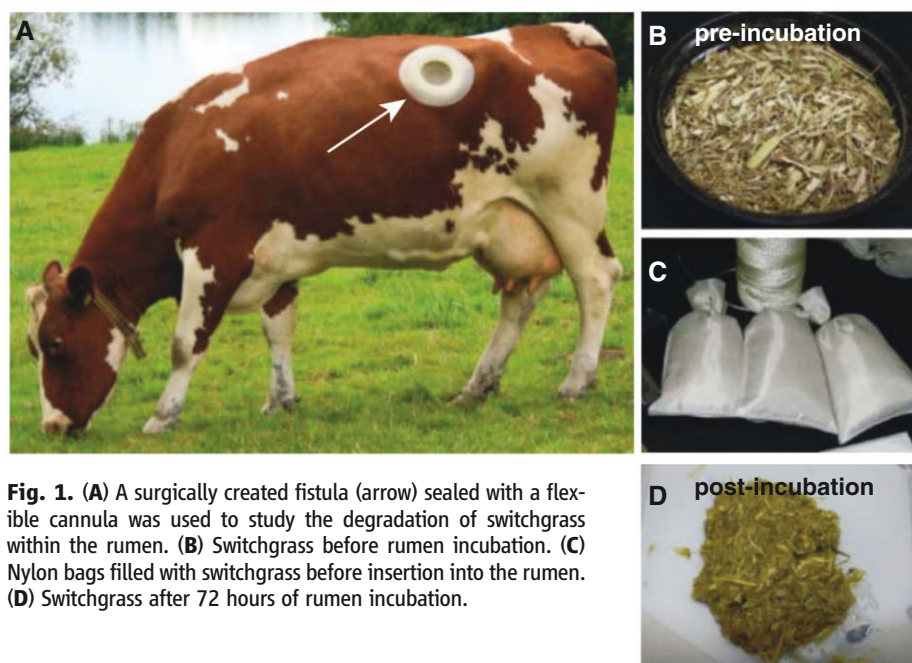
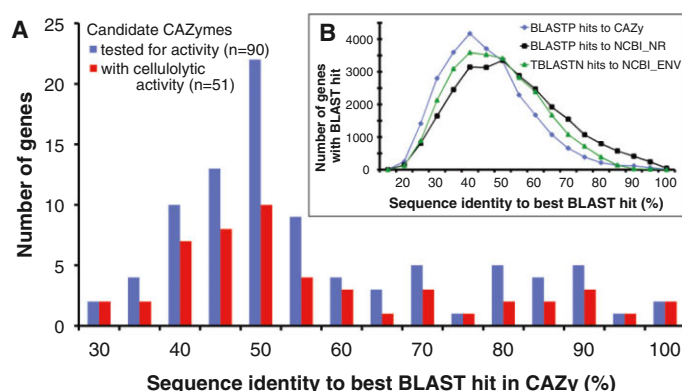


Fig. 1. (A) A surgically created fistula (arrow) sealed with a flexible cannula was used to study the degradation of switchgrass within the rumen. (B) Switchgrass before rumen incubation. (C) Nylon bags filled with switchgrass before insertion into the rumen. (D) Switchgrass after 72 hours of rumen incubation.

Fig. 2. (A) Sequence identity of 90 candidate sequences assembled from the switchgrass-associated rumen microbiome and tested for carbohydrate-degrading activity to known carbohydrate-active enzymes. Sequence identity to known enzymes is shown for tested candidates (blue) and candidates found to be active (red) toward at least one of the substrates used in the activity assays. (B)

Similarity distribution of CAZyme candidates ($n = 27,755$) containing a catalytic domain (CD) associated with carbohydrate hydrolytic activity or a carbohydrate-binding module (CBM). Sequences were compared to the CAZy (blue, 25,947 hits), NCBI-nr (black, 26,679 hits), and NCBI-env (green, 26,030 hits) databases (best BLAST hit, E-value $\leq 1e-5$); 482 genes contained both a CD and CBM, whereas 23,804 and 3469 genes contained only a CD or CBM, respectively.



didate gene sequences, with isolated examples of domains that are characteristic for eukaryotes.

Comparison of the 268 Gbp obtained by sequencing of the switchgrass-adherent microbiome to data from previously published lower-depth metagenomic studies of other plant-feeding animals (8, 10, 11) revealed that the number of candidate carbohydrate-active genes identified in the present study was larger by a factor of 5 than the combined number of candidate carbohydrate-active genes from all previous studies (table S5). The total amount of sequence analyzed in these earlier studies (combined: 0.21 Gbp) was three orders of magnitude less than the present data set and thus resulted predominantly in identification of partial genes. In contrast, genes in the present study are derived from assemblies with an average of 56-

fold sequence coverage, and more than 15,000 of the candidate carbohydrate-active enzymes reported here were predicted to represent full-length genes. Rarefaction analysis indicates that even at the considerable sequencing depth of this study, only a subset of genes present in the cow rumen microbiota was assembled (figs. S4 and S5).

Although the present study focuses on the validation of a subset of carbohydrate-active enzyme families, we expect the full repertoire of genes involved in biomass deconstruction to be present in the fiber-adherent rumen metagenomic data set. To test this hypothesis, we searched our data set for cohesins and dockerins, proteins commonly involved in the formation of lignocellolytic multi-enzyme complexes (cellulosomes) (20), and cellobiose phosphorylases, proteins belonging

to the family of glycosyltransferases. We were able to identify 80 and 188 ORFs containing the cohesin- and dockerin-specific PFAM domains, respectively. We also identified 811 genes from the switchgrass-adherent rumen microbiome that had significant similarity to cellobiose phosphorylases deposited in NCBI-nr (BLAST search, $E \leq 1e-5$). These results indicate that a wide spectrum of biomass-degrading genes can be identified through analysis of the sequence data generated in this study.

Focusing on our set of 27,755 predicted carbohydrate-active enzymes, we compared their sequences to entries in the Carbohydrate Active Enzyme (CAZy) database, which contains both experimentally verified and inferred carbohydrate-active enzymes (21). In the CAZy database, 1075, 1199, and 251 entries are annotated as β -(1,4)

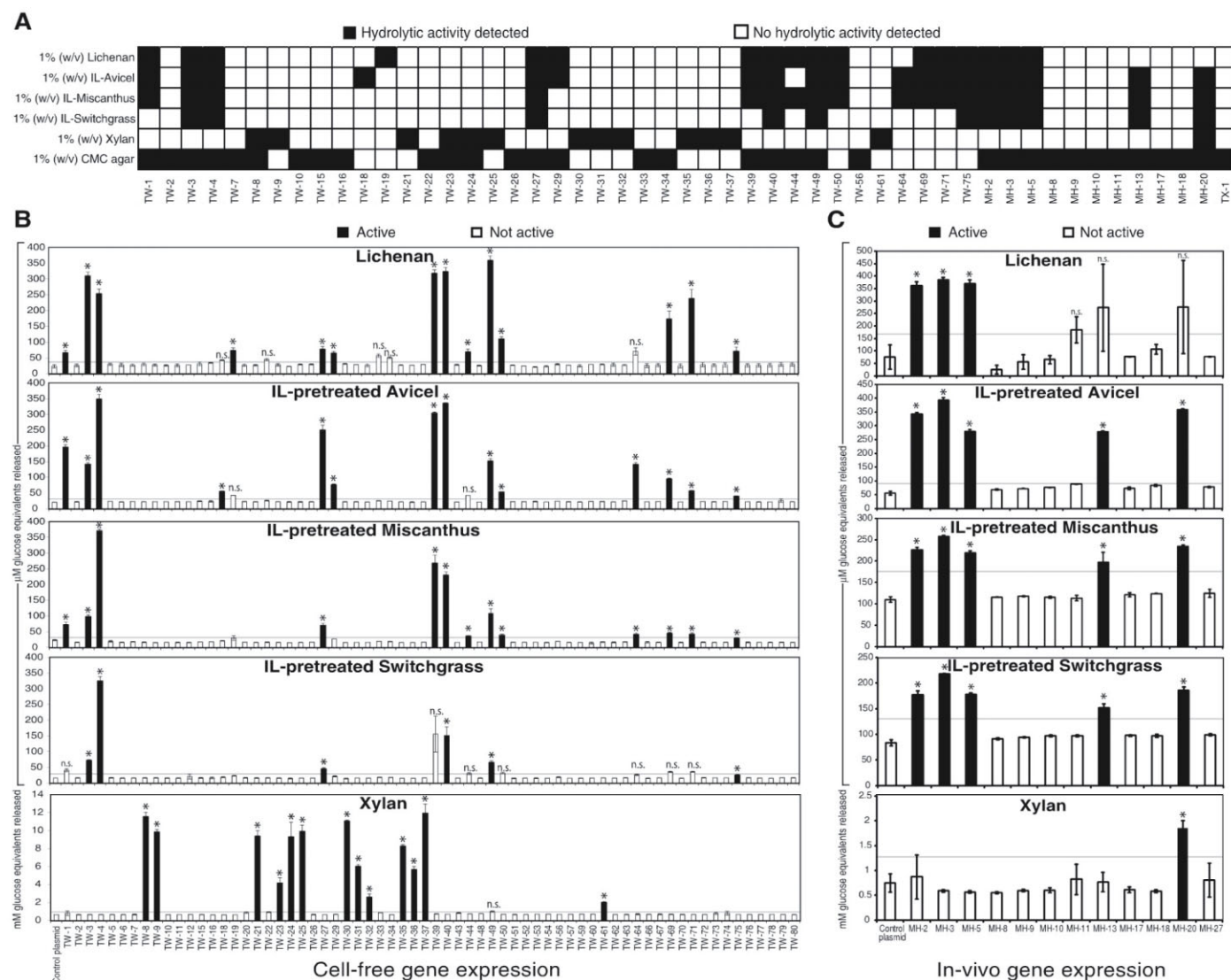


Fig. 3. Carbohydrolytic potential of candidate carbohydrate-active enzymes on glycosidic substrates of different complexity. **(A)** Summary of carbohydrate activities of 90 tested candidates on ten substrates. Candidates that were not active on any of the tested substrates and the four substrates that were recalcitrant to all tested candidates are not shown. **(B and C)** Cellulolytic activities of candidate carbohydrate-active enzymes on five substrates. Carbohydrate-active gene candidates were expressed in a cell-free system (B)

or using *Escherichia coli* as expression host (C). Samples were only considered "active" (■) if the measured mean glucose equivalent quantitatively exceeded the activity of negative controls plus one standard deviation by at least 50% (indicated by shaded horizontal line in each panel) and was significantly higher ($P < 0.05$, Student's *t* test) than the negative controls. Samples not meeting both criteria were considered as "not active" (□; n.s. = not significant). All measurements were performed in duplicate. IL, ionic liquid.

endoglucanases, β -glucosidases, and cellobiohydrolases, respectively (63%, 86%, and 87% of these entries lack an Enzyme Commission (EC) number, indicating that their assigned activity has not been verified biochemically). In our rumen-derived data set, we identified 1086, 1477, and 153 sequences whose most significant matches (BLAST search, $E \leq 1e-5$) were to a β -(1,4) endoglucanase, β -glucosidase, or cellobiohydrolase, respectively, within the CAZy database. Only 1% of these 2716 sequences were highly similar (>95% sequence identity) to any CAZy database entry, indicating that nearly all of these enzymes had not been previously deposited in CAZy. The overall lower efficiency at which new candidate cellobiohydrolases were identified may be due to their underrepresentation in the reference database, but even in this category we observed a 56% increase of candidate sequences with <95% sequence identity to sequences previously deposited in the CAZy database.

Conventional sequence homology-based enzyme discovery introduces a bias toward the identification of candidates similar to known enzymes, rather than new enzymes with low sequence identity and potentially divergent physicochemical properties. To assess our ability to discover carbohydrate-active enzymes with limited overall sequence identity to known proteins, we compared the amino acid sequences of the 27,755 putative carbohydrate-active genes identified in our metagenomic data set to all genes deposited in the NCBI nonredundant (NCBI-nr) and environmental database (NCBI-env) and to all 85,740 carbohydrate-active enzymes deposited in CAZy. Only 12% of the 27,755 carbohydrate-active genes assembled from the rumen metagenome were more than 75% identical to genes deposited in NCBI-nr, whereas 43% of the genes had less than 50% identity to any known protein (Fig. 2B).

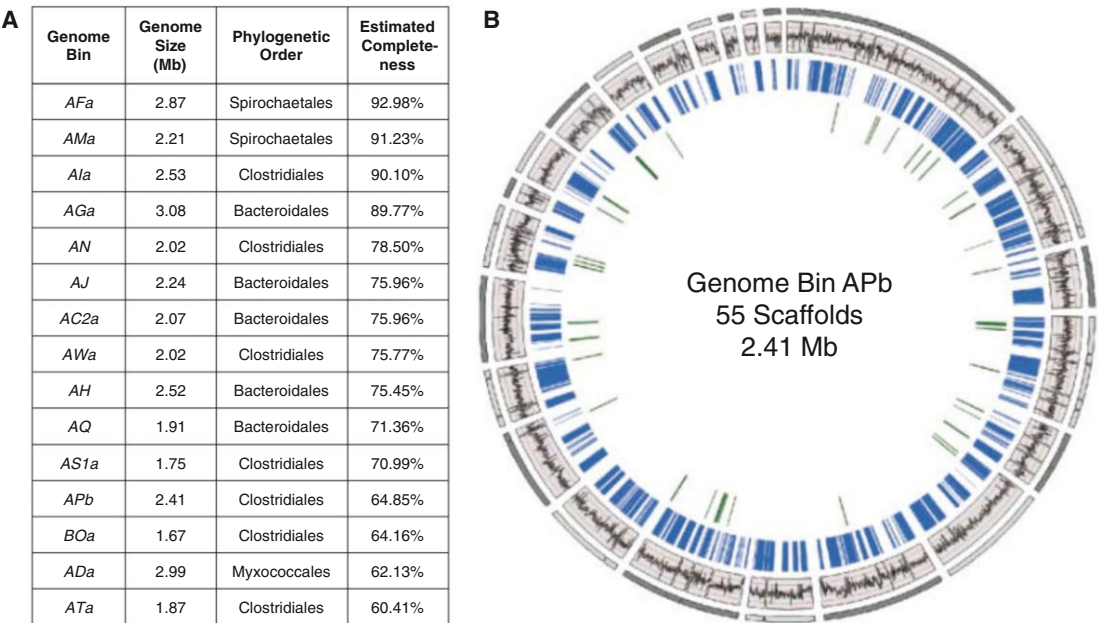
Twenty-four percent of our candidate carbohydrate-active genes were most similar to sequences annotated as “hypothetical protein” or “predicted protein” in NCBI-nr. Moreover, only 5% of our carbohydrate-active enzyme sequences are more than 75% identical to sequences deposited in the NCBI-env database (Fig. 2B and fig. S6), demonstrating that these enzymes also have not been observed in previous metagenome projects. These results reveal the abundance and diversity of putative carbohydrate-active enzymes in the fiber-adherent rumen microbiome.

To examine the validity of gene assemblies from short-read sequence data, we experimentally investigated a random subset ($N = 233$) of the putative rumen carbohydrate-active genes. We designed gene-specific primer pairs for each of these predicted carbohydrate-active genes and attempted to amplify the corresponding targets from the same DNA used to generate the metagenomic data. Using a single set of amplification conditions, we obtained polymerase chain reaction (PCR) products with the predicted size for 158 of 233 (68%) candidate genes. A randomly selected subset of the PCR products was further validated by sequencing, which confirmed in nearly all cases the computationally predicted sequence of the assembled candidate genes (>95% sequence identity, 28 of 29 putative genes). These results suggest that a substantial proportion of the genes predicted on the basis of short-read assemblies extracted from the metagenomic data represent authentic genes present in rumen microbes.

To evaluate the biochemical activity of the putative carbohydrate-active genes identified by metagenomic sequencing of the switchgrass-associated microbiome, we chose 90 candidate genes predicted to contain a glycoside hydrolase family 3, 5, 8, 9, 10, 26, or 48 domain or a

carbohydrate-binding module. The selected candidate genes were expressed using two complementary expression systems, and the obtained proteins were subjected to biochemical activity assays. The genes selected for expression ranged from 29 to 96% amino acid sequence identity to known carbohydrate-active proteins, with an average of less than 55% identity (Fig. 2A). We tested all 90 proteins for enzymatic activity on a panel of 10 different substrates. This panel included eight model substrates—carboxymethyl cellulose (CMC), *p*-nitrophenyl β -glucoside, gum guar, lichenan, laminarin, mannopentose, Avicel, and xylan—along with two potential biofuel feedstocks, miscanthus and switchgrass (22), to provide an initial understanding of the substrate specificity for each of the tested candidates. The biofuel crop substrates and Avicel were subjected to ionic liquid pretreatment before conducting the activity assays. In total, 51 of 90 (57%) tested proteins showed enzymatic activity against at least one of the substrates, suggesting that the candidate genes predicted by our metagenomic strategy are highly enriched in enzymes with relevant activities (Fig. 3 and table S6). There was no evidence that proteins with high sequence identity to known enzymes were more likely to be active than proteins with low sequence similarity ($P = 0.66$, Kolmogorov-Smirnov test; Fig. 2A). Inactivity of the remaining carbohydrate-active candidates in these assays could be due to a number of reasons, including false-positive prediction of carbohydrate-active enzyme domains, minimal expression and/or misfolding of candidate proteins, or suboptimal reaction conditions. The overall high validation rate observed in these assays suggests that the number and sequence diversity of known genes encoding hydrolytic enzymes from these and possibly other enzyme

Fig. 4. (A) Draft genomes assembled from switchgrass-adherent rumen microbes. Completeness was estimated as fraction of the number of identified and the number of expected core genes within the phylogenetic order. For more information on the assembled genomes, see table S10. **(B)** Circular representation of the draft genome (genome bin APb) validated by single amplified genome analysis. From outside toward the center: outermost circle, scaffolds within the draft genome (random order, low-quality regions removed); circle 2, Illumina read coverage in metagenomic data (each horizontal red line indicates 25-fold coverage); circle 3 (blue tick marks), regions of the draft genome simultaneously covered by 454 reads derived from a single amplified genome; innermost circle (green tick marks), location of glycoside hydrolase genes on draft genome.



families was substantially increased through this metagenomic data set.

A considerable fraction (20%) of the tested carbohydrate-active enzyme candidates showed activity toward biofuel crops pretreated with ionic liquids, one of the most promising initial steps in the deconstruction of biomass (23). Because ionic liquids can inhibit enzymatic biomass degradation (24), the retention of enzymatic activity in their presence makes these proteins promising candidates for more detailed physicochemical analyses. In addition, the tested set of target candidates also included two enzymes (MH-9 and MH-10) that showed activity on CMC agar plates and contained only a carbohydrate-binding module, but no known catalytic domain specific for carbohydrate-active enzymes (table S6). It is possible that these two enzymes contain catalytic domains that share little similarity with the catalytic domains of currently known carbohydrate-active enzymes.

To enable genomic studies of these microbes, we developed a strategy for producing draft genomes from deep metagenomic data. An initial assembly of the 268 Gbp of metagenomic sequence resulted in 179,092 scaffolds, of which the 65 largest ranged in size from 0.5 to 1.5 Mbp (tables S8 and S9). Only 47 (0.03%) of the assembled scaffolds showed high levels of similarity ($\geq 90\%$ identity over ≥ 1000 bp) to previously sequenced genomes available in GenBank. Most of these alignable scaffolds were small (median length: 1626 bp) and the aligned regions typically covered nearly the entire length of the scaffold (median: 91% of scaffold length). These results suggest that the vast majority of the assembled scaffolds represent segments of hitherto uncharacterized microbial genomes. We further validated these assemblies via two independent indicators of scaffold integrity: (i) level and uniformity of read depth in subregions, and (ii) mate-pair support. We identified 26,042 scaffolds greater than 10 kbp that satisfied these criteria for scaffold integrity (18), totaling 568 Mbp (N50: 24 kbp; longest scaffold: 541 kbp). To generate draft genomes, we binned the validated scaffolds by means of two complementary properties expected to be present in scaffolds derived from the same genome: (i) tetranucleotide frequencies (TNFs) and (ii) read coverage. TNF signatures are generally an effective approach for distinguishing sequences derived from different genomes but can be similar for closely related species (25, 26). In contrast, read coverage is directly correlated to the relative abundance of each organism in the sample and can thus be used to distinguish scaffolds that are likely derived from different closely related organisms. In total, 446 genome bins with consistent TNF and read coverage were formed. To estimate the completeness of the largest potential microbial draft genomes identified through this approach, we first determined the most likely phylogenetic order from which each of these bins was derived. For each of these orders, we used all available sequenced reference genomes (table

S11) to identify a minimal set of core genes that are present in all members of this order (27, 28). Comparison of each draft genome to the pan-genome of the respective phylogenetic order demonstrated that between 60% and 93% of the core genes were included in the 15 draft genomes found to be most complete by this measure, similar to the fraction found in each reference genome used for comparison (Fig. 4A and tables S10 and S12). These observations suggest that near-complete draft genomes were successfully assembled. To address the possibility that the completeness of individual draft genomes was overestimated as a result of binning of scaffolds derived from multiple organisms, we further validated their authenticity by copy number analysis of genes that were present only in single copy in all reference genomes of the respective phylogenetic order (18) (tables S10 and S12).

To test experimentally the validity and completeness of draft genomes derived from metagenomic scaffold bins, we obtained genome sequence data from individual uncultured microbial cells isolated directly from the same complex rumen community. Single cells loosely adherent to switchgrass were isolated using fluorescence-activated cell sorting (29) followed by whole genome amplification (30). Screening of 16S sequences suggested that one of the single cells analyzed was related to the fibrolytic *Butyrivibrio fibrisolvens* and matched bin APb, one of the largest bins assembled from metagenomic data (Fig. 4A and table S10). From this particular single cell, we generated 65,272 reads (22.5 Mbp after appropriate filtering) of which 55% mapped to genome bin APb. The remaining mappable single-cell reads matched either unbinned scaffolds or assembly regions with poor scaffold integrity. Each of the 55 scaffolds in bin APb was supported by substantial numbers of mapped single cell-derived reads, suggesting that all scaffolds in bin APb represent segments of the genome of the same single organism (Fig. 4B). These results directly support the assumption that individual genome bins derived from our assembly represent authentic draft genomes and suggest that substantial proportions of the respective genomes are covered by these bins.

Discovery of full-length genes with defined functions from complex microbial communities has previously been severely limited by the low throughput of the required cellular and molecular manipulations (8, 11, 31). Our study demonstrates the potential of deep sequencing of a complex community to accurately reveal genes of interest at a massive scale, and to generate draft genomes of uncultured novel organisms involved in biomass deconstruction. Although this work focused on the identification and validation of new carbohydrate-active enzymes, these data sets provide an extensive resource for the discovery of a multitude of other classes of enzymes known to exist in the rumen, and the general approach presented here will be applicable to other environmental microbial communities.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/331/6016/463/DC1
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Cleavage of NIK by the API2-MALT1 Fusion Oncoprotein Leads to Noncanonical NF- κ B Activation

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Proper regulation of nuclear factor κ B (NF- κ B) transcriptional activity is required for normal lymphocyte function, and deregulated NF- κ B signaling can facilitate lymphomagenesis. We demonstrate that the API2-MALT1 fusion oncoprotein created by the recurrent t(11;18)(q21;q21) in mucosa-associated lymphoid tissue (MALT) lymphoma induces proteolytic cleavage of NF- κ B-inducing kinase (NIK) at arginine 325. NIK cleavage requires the concerted actions of both fusion partners and generates a C-terminal NIK fragment that retains kinase activity and is resistant to proteasomal degradation. The resulting deregulated NIK activity is associated with constitutive noncanonical NF- κ B signaling, enhanced B cell adhesion, and apoptosis resistance. Our study reveals the gain-of-function proteolytic activity of a fusion oncoprotein and highlights the importance of the noncanonical NF- κ B pathway in B lymphoproliferative disease.

Mucosa-associated lymphoid tissue (MALT) lymphoma, the most common extranodal B cell tumor, accounts for 8% of

minus of API2 [also termed cellular inhibitor of apoptosis 2 (cIAP2)] linked to the C terminus of MALT1 (7). Wild-type MALT1 mediates antigen-induced nuclear factor κ B (NF- κ B) stimulation, which leads to lymphocyte survival and proliferation (2). MALT1 activates canonical NF- κ B signaling after autoligomerization induced by upstream

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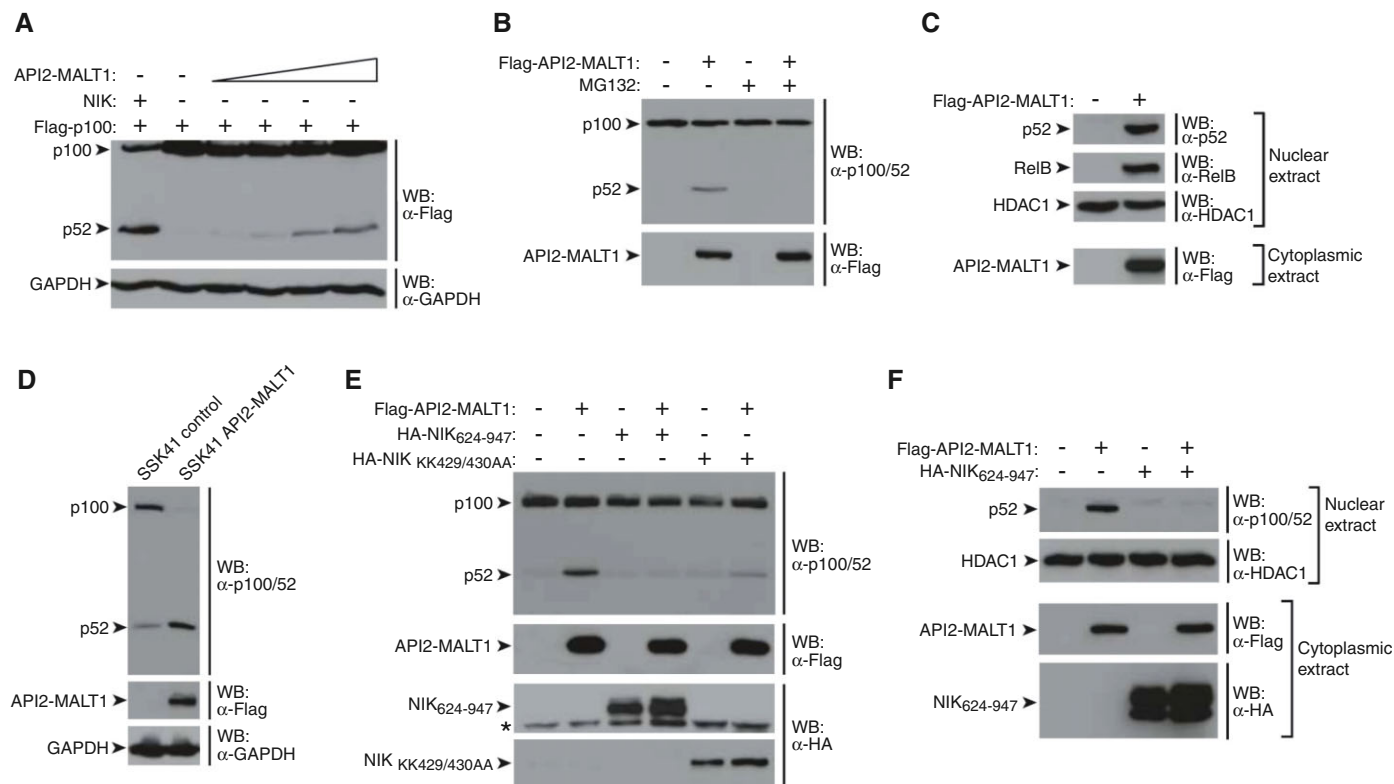


Fig. 1. API2-MAL11 induces noncanonical NF- κ B signaling through NIK. **(A and B)** HEK293T cells were transfected as indicated, and p100 processing to p52 was assessed by Western blot (WB) with an antibody against Flag (α -Flag) (A) or α -p100/52 (to detect endogenous p100/52) (B). Where indicated, cells were treated with proteasome inhibitor, MG132. **(C)** After transfection of HEK293T cells, nuclear extracts were prepared and analyzed for p52 and RelB by WB. **(D)** p100 processing in

lysates from control SSK41 cells or SSK41 cells stably expressing API2-MALT1 was analyzed by WB. **(E and F)** HEK293T cells were transfected with API2-MALT1 in the absence or presence of NIK mutants. Endogenous p100 processing was analyzed by WB (**E**), or nuclear extracts were analyzed for the presence of p52 (**F**). *Nonspecific band. HDAC1, histone deacetylase 1 (loading control for nuclear extract). Data are representative of at least three separate experiments.

factors CARMA1 and Bcl10 (3, 4). It is thought that because the API2 moiety mediates autooligomerization, API2-MALT1 can stimulate NF- κ B independent of upstream signals (5, 6). This may explain why t(11;18)-positive MALT lymphomas are not dependent on antigenic stimulation for progression, whereas t(11;18)-negative tumors require ongoing chronic inflammation for survival. The phenomenon is best exemplified by gastric MALT lymphomas, the majority of which arise in the setting of chronic *Helicobacter pylori* gastritis and are cured by eradication of *H. pylori* with antibiotics. In contrast, t(11;18)-positive gastric tumors are resistant to this treatment and are associated with advanced-stage disease (1).

We discovered that besides activating canonical NF- κ B, expression of API2-MALT1 in human embryonic kidney 293T (HEK293T) or the human B lymphoma (SSK41) cells induced proteasome-dependent processing of the NF- κ B precursor, p100, to its mature form, p52, and stimulated nuclear translocation of p52/RelB (Fig. 1, A to D, and fig. S1) (7). These findings indicate that API2-MALT1 activates the “noncanonical” NF- κ B pathway, which requires NF- κ B-inducing kinase (NIK)-dependent phosphorylation and activation of inhibitor of NF- κ B (κ B) kinase- α (IKK α).

This, in turn, triggers proteasome-mediated partial degradation of p100 to p52 and generation of transcriptionally active p52/RelB NF- κ B dimers (8). Consistent with this notion, dominant-negative NIK mutants (9, 10) blocked API2-MALT1-dependent p100 processing and p52 nuclear translocation (Fig. 1, E and F).

cIAP1 and cIAP2 (API2) associate with NIK and promote NIK degradation via RING domain ubiquitin ligase activity (11–13). We hypothesized that API2-MALT1, which lacks the cIAP2 RING domain, stimulates noncanonical signaling through competitive inhibition of cIAP-mediated NIK degradation. In testing this, we discovered that expression of API2-MALT1 instead induced proteolytic cleavage of NIK, generating ~37-kD N-terminal and ~70-kD C-terminal NIK fragments (Fig. 2, A and B, and fig. S2).

API2-MALT1 fusion transcripts invariably contain three intact baculoviral IAP repeat (BIR) domains from API2 and an intact “caspaselike” domain from MALT1, which suggests that these domains are critical for lymphomagenesis (1). The caspaselike domain of wild-type MALT1 has proteolytic activity, and Bcl10 and the NF- κ B inhibitor, A20, are the only known substrates (14, 15). We therefore investigated whether the cas-

paselike domain within API2-MALT1 is also able to cleave NIK. Deletion mutants of API2-MALT1 lacking portions of the caspaselike domain and API2-MALT1-C678A (16), in which the catalytic cysteine within the MALT1 proteolytic domain is replaced with alanine, were unable to induce NIK cleavage (Fig. 2C and fig. S3, A to C). Furthermore, treatment with z-Val-Arg-Pro-Arg-fluoromethylketone (z-VRPR-fmk), a MALT1 protease inhibitor (15), blocked API2-MALT1-induced NIK cleavage, whereas z-IETD-fmk, a caspase-8 inhibitor, had no effect (fig. S3, D and E). Finally, an in vitro cleavage reaction using purified recombinant proteins showed that NIK is a direct substrate of the MALT1 protease domain (Fig. 2D and fig. S4).

Cellular expression of the MALT1 moiety alone was unable to induce NIK cleavage, which suggests that the API2 moiety also contributes in some way (Fig. 2E). Indeed, analyses revealed that NIK physically associates with API2-MALT1 via the API2 moiety (Fig. 2F) and that the region within the API2 moiety that mediates auto-oligomerization of API2-MALT1 (amino acids 49 to 98) (5) is required for efficient API2-MALT1-dependent NIK cleavage and p100 processing (fig. S5). The collaborative relationship of the

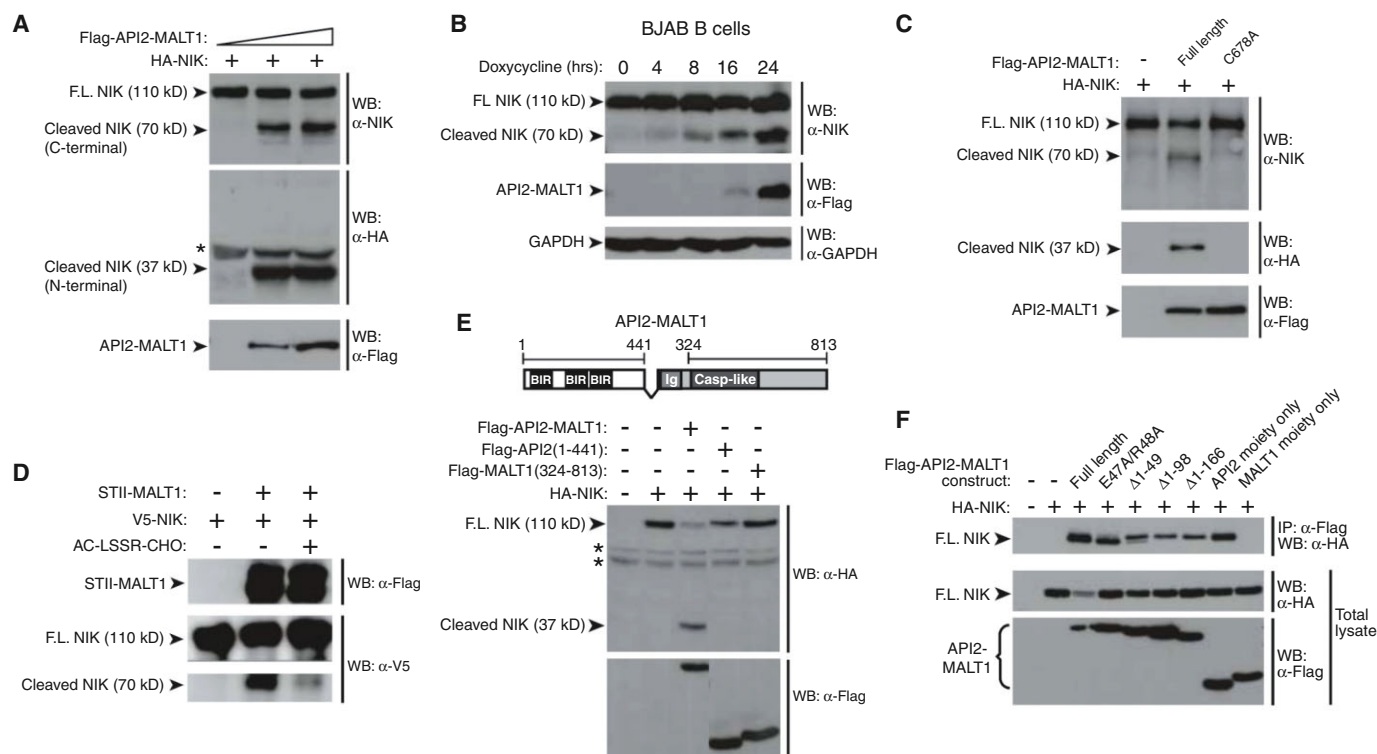


Fig. 2. API2-MALT1 induces NIK cleavage, a phenomenon requiring both the API2 moiety and MALT1 protease activity. **(A)** HEK293T cells were transfected as indicated, and NIK cleavage fragments were detected by WB. **(B)** BJAB cells expressing API2-MALT1 from a tetracycline-inducible promoter were treated with doxycycline. WB with an antibody raised against a C-terminal NIK sequence revealed time-dependent generation of an endogenous 70-kD NIK cleavage fragment. To enhance detection of full-length (FL) NIK, cells were incubated with 25 μ M MG132. **(C)** HEK293T cells were transfected as indicated, and the presence of the N-terminal 37-kD and the C-terminal 70-kD

NIK cleavage fragments was analyzed by WB. **(D)** Recombinant purified NIK-V5-bioC and StrepII-Flag-tagged MALT1 were incubated in kosmotropic salt buffer for 6 hours at 37°C, with or without 100 μ M MALT1 protease inhibitor AC-LSSR-CHO, and analyzed by WB. **(E)** HEK293T cells were transfected as indicated, and the 37-kD NIK cleavage fragment was detected by WB. **(F)** HEK293T cells were transfected, and immunoprecipitations were carried out using α -Flag-agarose. For a detailed description of API2-MALT1 mutants, see the legend to fig. S5. *Nonspecific band. Data are representative of at least three separate experiments.

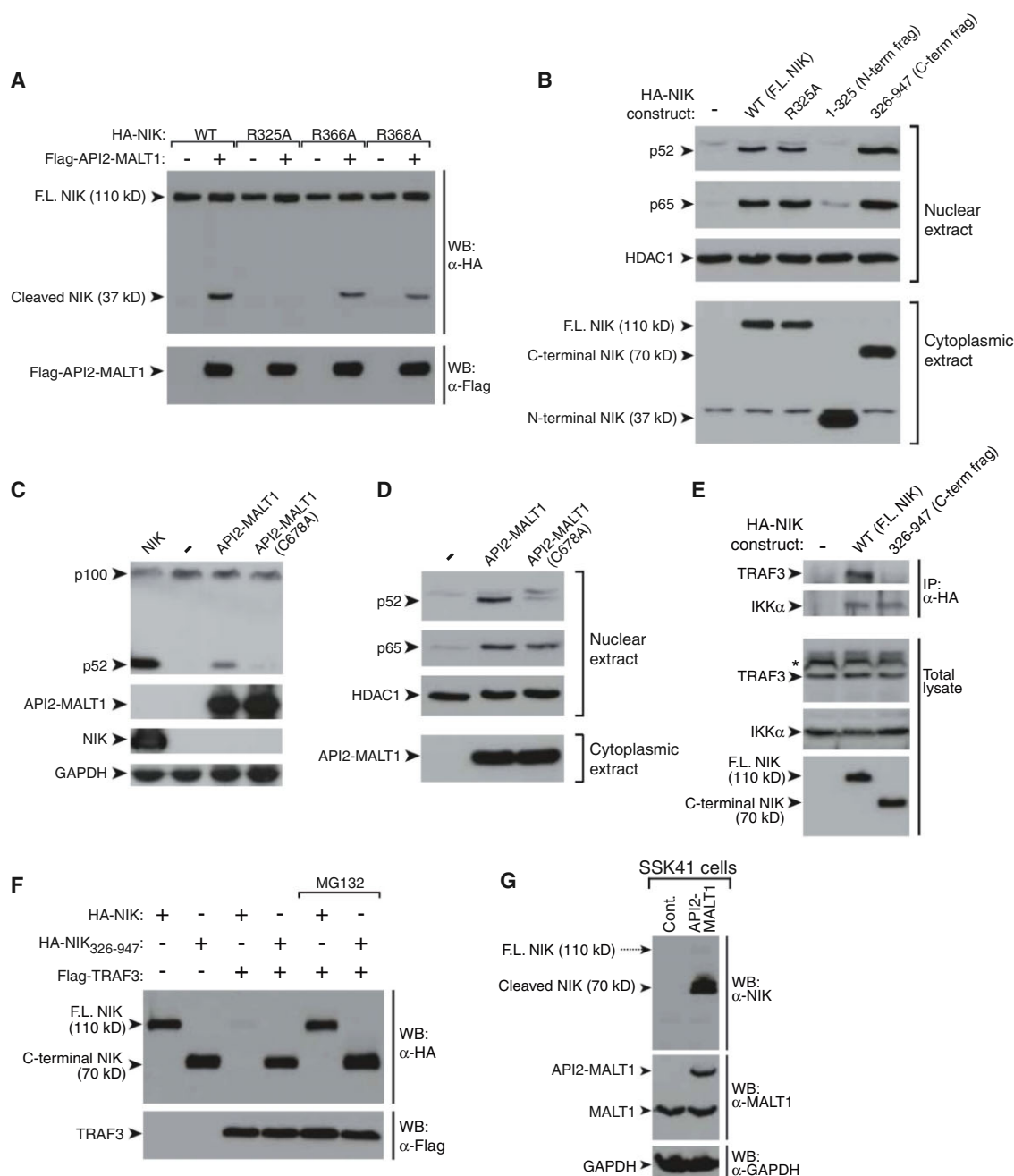
API2 and MALT1 moieties in achieving NIK cleavage was further underscored in several different contexts. First, unlike API2-MALT1, induced expression of wild-type MALT1 in BJAB B cells did not result in NIK cleavage (fig. S6A). Second, NIK cleavage was not observed in SSK41 B cells, which are characterized by *MALT1* gene amplification, overexpression of MALT1, and constitutive MALT1 protease activity (14, 17, 18). In contrast to A20, NIK was cleaved only if SSK41 cells were engineered to express API2-MALT1 (fig. S6B). Third, coexpression of wild-type MALT1 with Bcl10, which triggers MALT1 oligomerization and activation (3), did lead to cleavage of A20 but not of NIK (fig. S6, C and D). Fourth, ligand-induced B cell receptor stimulation, which sim-

ilarly activates the MALT1 protease (14), did not trigger NIK cleavage (fig. S6E). Furthermore, although MALT1 oligomerization and activation require Bcl10 (3), API2-MALT1-mediated NIK cleavage occurred in the absence of Bcl10 (fig. S6F). Together, these findings suggest that NIK is a substrate for the MALT1 protease domain, but only when this domain is present within the context of API2-MALT1.

Structural analyses predict that the MALT1 protease should show specificity for substrates with a basic or uncharged residue at P1 (N-terminal to the cleavage site) (19). Furthermore, the MALT1 cleavage sites of Bcl10 and A20 both contain a P2-serine preceding a P1-arginine (14, 15, 20). Thus, we identified candidate P2-Ser/P1-Arg MALT1

cleavage sites within NIK that would generate fragments of ~37 and 70 kD (fig. S7A), and we individually changed each candidate P1-Arg to Ala. The R366A and R368A NIK mutants were readily cleaved by API2-MALT1; however, the R325A mutant was resistant (Fig. 3A), which suggests that API2-MALT1-dependent cleavage of NIK occurs at R325 (fig. S7B). Expression of the resulting C-terminal NIK cleavage fragment, NIK(326–947), which retains the kinase domain, induced robust p100 processing (fig. S7C), p52 nuclear translocation (Fig. 3B), and noncanonical NF- κ B target gene expression (fig. S7D). NIK(326–947) also induced nuclear translocation of the p65 NF- κ B subunit, which indicated activation of the canonical NF- κ B pathway

Fig. 3. API2-MALT1-dependent cleavage of NIK at R325 generates an active C-terminal fragment. **(A)** HEK293T cells were transfected as indicated, and the 37-kD N-terminal NIK cleavage fragment was detected by WB. **(B)** HEK293T cells were transfected as indicated, and nuclear translocation of p52 and p65 NF- κ B subunits was assessed. **(C and D)** HEK293T cells were transfected as indicated, and endogenous p100 processing (C) and nuclear translocation of NF- κ B subunits (D) were assessed. **(E)** HEK293T cells were transfected as indicated, and the ability of endogenous TRAF3 or IKK α to immunoprecipitate with each NIK protein was assessed. **(F)** HEK293T cells were transfected as indicated and then incubated in the absence or presence of 25 μ M MG132. The presence of NIK was detected by WB. **(G)** Cell lysates were prepared in the absence of MG132 and analyzed by WB to detect full-length (F.L.) NIK and the 70-kD C-terminal NIK cleavage fragment. Data are representative of at least three separate experiments.



as well (Fig. 3B). Conversely, API2-MALT1-C678A, the catalytically inactive mutant that cannot induce NIK cleavage to produce NIK(326–947), failed to stimulate p100 processing (Fig. 3C) and p52 nuclear translocation (Fig. 3D and fig. S8). NIK associates with the adaptor protein, TRAF3, via an N-terminal NIK domain (amino acids 78 to 84), and this interaction targets NIK for proteasomal degradation (21–23). Because cleavage of NIK at R325 separates this TRAF3-binding site from the NIK kinase domain, we hypothesized that the active C-terminal NIK cleavage product would be resistant to TRAF3-directed

degradation. Indeed, NIK(326–947) retained binding to IKK α but not to TRAF3 (Fig. 3E) and, unlike full-length NIK, was resistant to TRAF3-dependent proteasomal degradation (Fig. 3F). We also demonstrated the unique stability of the API2-MALT1-generated C-terminal NIK cleavage fragment in SSK41 B lymphoma cells. In the absence of MG132 (a proteasome inhibitor), expression of full-length NIK was very low, regardless of whether API2-MALT1 was present, consistent with the fact that NIK is subject to constitutive proteasomal degradation (21, 24) (Fig. 3G). In contrast, high levels of

endogenous C-terminal NIK cleavage fragment were detected in SSK41 cells expressing API2-MALT1 (Fig. 3G).

API2-MALT1-dependent generation of the C-terminal NIK cleavage fragment in SSK41 cells was associated with enhanced transcription of noncanonical NF- κ B target genes, including *Pim-2*, an oncogenic kinase that blocks apoptosis by phosphorylating the proapoptotic Bcl-2 family member BAD (fig. S9, A and B) (25–29). RNA interference-mediated knockdown of NIK in API2-MALT1-expressing SSK41 cells led to loss of the 70-kD NIK fragment, loss of p100

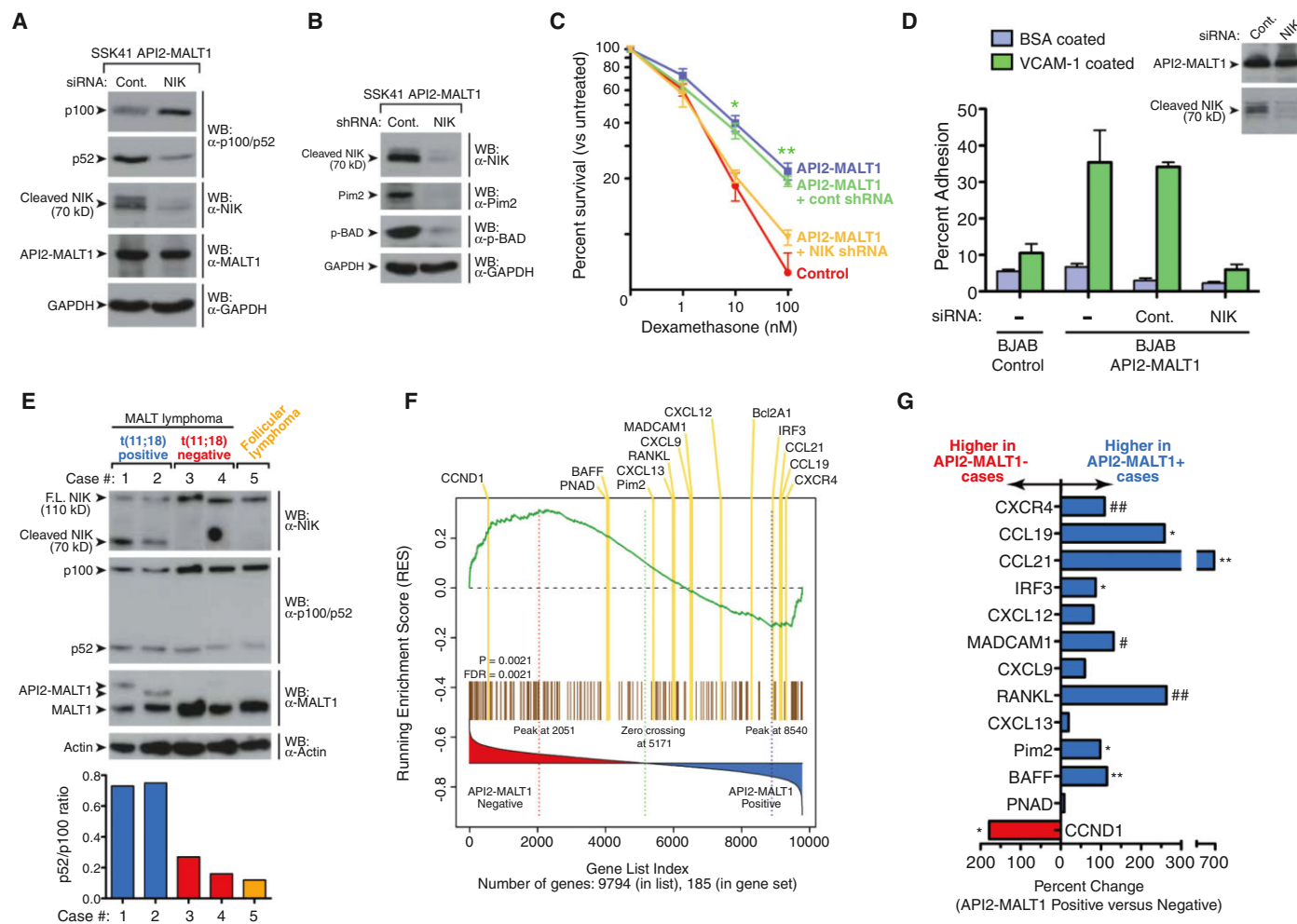


Fig. 4. API2-MALT1-dependent NIK cleavage is associated with up-regulation of noncanonical NF- κ B target genes, results in an altered B cell phenotype, and occurs in t(11;18)-positive MALT lymphoma. (A) API2-MALT1-expressing SSK41 cells were transiently transfected with control or NIK small interfering RNA (siRNA), and p100 processing was assessed by WB. (B and C) API2-MALT1-expressing SSK41 cells were stably infected with control or NIK small hairpin RNA (shRNA) lentiviral particles. The 70-kD NIK cleavage fragment, Pim-2, and phospho-Ser¹¹²-BAD levels were compared by WB (B). Cells were treated for 48 hours with dexamethasone, and percent viability was compared (C). Data are expressed as average $\pm$ SEM for four separate experiments. * P < 0.005, ** P < 0.0001. (D) API2-MALT1-expressing BJBAB cells were transfected with control or NIK siRNA, and adhesion to VCAM-1-coated plates was assessed. Error bars represent SEM of duplicate determinations. Data are representative of three separate experiments. (E) Expression of API2-MALT1 in two t(11;18)-positive

MALT lymphoma samples was confirmed by WB. p52/p100 ratios were quantified using densitometry. Both t(11;18)-positive cases had the same breakpoint in the *API2* gene (between exons 7 and 8) but different breakpoints in the *MALT1* gene [between exons 4 and 5 (case 1) and exons 6 and 7 (case 2)]. A follicular lymphoma and two t(11;18)-negative MALT lymphoma tumor specimens were used as controls. (F) GSEA of NF- κ B target genes was performed in a set of MALT lymphomas with t(11;18) versus those without translocation from tumor collection no. 1 (34). The distribution of the genes is listed according to rank position, and known noncanonical NF- κ B gene targets are highlighted in yellow. Absolute enrichment gave P = 0.0021 and false discovery rate (FDR) = 0.0021. (G) Comparison of noncanonical target gene expression in t(11;18)-positive versus negative cases from tumor collection no. 2 (34). Statistical testing for genes differentially expressed between the two types of MALT lymphomas was done by *t* test. * P < 0.05, ** P < 0.01, * P < 0.005, ** P < 0.001.

processing, and loss of API2-MALT1-dependent induction of Pim-2 kinase and BAD phosphorylation (Fig. 4, A and B). In accordance with its effect on antiapoptotic signal transduction, API2-MALT1 expression protected SSK41 cells from dexamethasone-induced cell death, which was reversed by NIK knockdown (Fig. 4, B and C, and fig. S10). Knockdown of IKK α impaired API2-MALT1-dependent protection, which supports a role for the noncanonical NF- κ B pathway in mediating this effect of API2-MALT1-induced NIK cleavage (fig. S11). IKK β knockdown impaired API2-MALT1-dependent protection as well, which implies that the canonical pathway may also contribute (fig. S11).

We next investigated the impact of API2-MALT1-dependent NIK cleavage on B cell adhesion because we had observed that API2-MALT1 induced the expression of B cell integrins (fig. S9A), known noncanonical NF- κ B gene targets (27, 28). API2-MALT1 expression was associated with increased B cell adhesion to plates coated with the endothelial protein vascular cell adhesion molecule VCAM-1, and this proadhesive phenotype was fully dependent on NIK (Fig. 4D). Lymphocyte adhesion is thought to play a role in lymphoma dissemination, thus NIK-cleavage-dependent API2-MALT1-induced adhesion may contribute to the higher rate of tumor spread among t(11;18)-positive MALT lymphomas (1). Again, knockdown of IKK α or IKK β impaired API2-MALT1-dependent adhesion, which suggests that both noncanonical and canonical pathways contribute to the proadhesive phenotype after API2-MALT1-dependent NIK cleavage (fig. S12).

The striking pattern of NIK cleavage and stability observed in API2-MALT1-expressing B lymphoma cell lines was recapitulated in MALT lymphoma patient specimens. Full-length NIK levels were relatively low in all lymphomas, whereas an endogenous 70-kD C-terminal NIK fragment was detected only in t(11;18)-positive MALT lymphomas expressing API2-MALT1 (Fig. 4E). The presence of the NIK cleavage product was associated with an elevated p52/p100 ratio in the t(11;18)-positive tumors, which indicated enhanced noncanonical NF- κ B activation (Fig. 4E). We performed absolute gene set enrichment analysis (GSEA) comparing the expression of NF- κ B target genes between t(11;18)-positive MALT lymphomas ($n = 9$) and those with no translocation ($n = 8$). Analysis revealed a significant difference in the pattern of expression, with most known noncanonical NF- κ B target genes over-represented in the t(11;18)-positive cases (Fig. 4F, fig. S13, and tables S1 and S2). One exception is *cyclin D1*, although its categorization as a noncanonical NF- κ B target gene is controversial (30). We then compared the expression of these noncanonical target genes in a completely separate group of six t(11;18)-positive and eight t(11;18)-negative MALT lymphomas that were collected at a different institution and, again, found that many noncanonical NF- κ B target genes were

more highly expressed in the t(11;18)-positive tumors (Fig. 4G). *CXCR4*, which encodes a chemokine receptor whose expression is associated with widespread lymph node involvement in B lymphomas (31–33), is one intriguing example of a noncanonical gene target that is up-regulated in t(11;18)-positive tumors in both tumor collections (Fig. 4, F and G) (34).

Deregulated NIK activity has been increasingly implicated in the pathogenesis of B cell neoplasms. For example, an EFTUD2-NIK fusion oncoprotein that retains the NIK kinase domain—but lacks the TRAF3-binding site and is resistant to proteasomal degradation—was recently identified in a case of multiple myeloma (24). Another recent report described a murine model of B lymphoproliferative disease in which a NIK mutant lacking the TRAF3-binding domain (NIK Δ T3) was expressed in B cells (35). Compared with control transgenic mice expressing full-length NIK, the NIK Δ T3 mice demonstrated increased NIK levels with enhanced p100 processing in B cells. They also showed expanded MALT and profound splenic marginal zone B cell hyperplasia, a phenotype that bears similarity to the E μ -API2-MALT1 transgenic mouse (36). Together with our results, these findings suggest that separating the TRAF3-binding site on NIK from the kinase domain, either through aberrations of the NIK gene or through proteolytic cleavage of NIK protein, may represent a common mechanism for deregulating NIK activity in B cell neoplasms. Our findings suggest that in API2-MALT1-expressing MALT lymphomas, the API2 moiety mediates autoligomerization of API2-MALT1 and recruitment of NIK, and the MALT1 protease domain cleaves NIK, which leads to degradation-resistant NIK kinase and deregulated noncanonical NF- κ B signaling (see model, fig. S14). Data suggest that NIK cleavage protects API2-MALT1-expressing B cells from apoptosis and promotes B cell adhesion, both of which could contribute to the more aggressive phenotype of t(11;18)-positive MALT lymphomas (1). Disrupting the API2-NIK interaction and/or blocking MALT1 protease or NIK kinase activity could represent new treatment approaches for refractory t(11;18)-positive MALT lymphoma.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/331/6016/468/DC1
Materials and Methods
Figs. S1 to S14
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Intramembrane Cleavage of AMA1 Triggers *Toxoplasma* to Switch from an Invasive to a Replicative Mode

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Apicomplexan parasites invade host cells and immediately initiate cell division. The extracellular parasite discharges transmembrane proteins onto its surface to mediate motility and invasion. These are shed by intramembrane cleavage, a process associated with invasion but otherwise poorly understood. Functional analysis of *Toxoplasma* rhomboid 4, a surface intramembrane protease, by conditional overexpression of a catalytically inactive form produced a profound block in replication. This was completely rescued by expression of the cleaved cytoplasmic tail of *Toxoplasma* or *Plasmodium* apical membrane antigen 1 (AMA1). These results reveal an unexpected function for AMA1 in parasite replication and suggest that invasion proteins help to promote parasite switch from an invasive to a replicative mode.

Host cell invasion by *Toxoplasma* and *Plasmodium* involves discharge of secretory organelles called micronemes (1) and rhoptries. Apical membrane antigen 1 (AMA1), a microneme protein, is crucial for invasion (2) and is part of the moving junction complex formed during invasion (3). In *Toxoplasma gondii*, AMA1 and microneme protein-2 (MIC2), MIC6, MIC8, and MIC12 are cleaved during invasion within their transmembrane domain (TMD) by a rhomboid activity (4–7), releasing them from the parasite surface. In *Plasmodium*, the majority of AMA1 shedding is mediated by a subtilisin protease (6, 8, 9), but cleavage by a rhomboid protease also occurs (6). The role of AMA1 shedding is unknown. *Toxoplasma* encodes six rhomboids (10), two of which have been functionally dissected: ROM1 is expressed in the micronemes but does not play a crucial role in invasion (11, 12); ROM4 localizes to the plasma membrane (13, 14) and cleaves MIC2, AMA1, and MIC8 (15). *Plasmodium falciparum* ROM4 sheds the micronemal erythrocyte-binding protein EBA175 (16) and possibly other adhesins (17). After invasion, the parasite initiates replication within a parasitophorous vacuole. *Toxoplasma* tachyzoites divide by endodyogeny, in which repeated cycles of replication produce numerous new parasites equipped for egress and invasion. In contrast, *Plasmodium* replicates by schizogony, in which a multinucleated syncytium (schizont) is formed that undergoes cytokinesis only after completion of nuclear division. The signals governing initiation of replication are unknown.

Rhomboids are broad-substrate-specificity serine proteases that recognize helix-destabilizing

residues within the substrate's TMD (18). We reasoned that expression of a *T. gondii* ROM4 mutant, able to bind the substrate but unable to cleave it, would sequester the substrate from the endogenous protease and behave as dominant negative. To regulate expression of the protease, we used the FK506 binding protein destabilization domain (dd) system in which fusion proteins are degraded unless a ligand, Shld-1, is added (19). We expressed either a control wild-type (WT) construct (ddROM4) or a mutant form, in which the catalytic Ser⁴⁰⁹ was substituted with an Ala residue (ddROM4_{S-A}) (20). Similar mutations ablate rhomboid activity (13, 14, 17). To validate the system, a ROM1 mutant (ddROM1_{S-A}) was also generated. The transgenic proteins were correctly localized and expressed in a Shld-1-dependent manner (Fig. 1, A and B). Expression of ddROM4 was detectable 5 min after Shld-1 treatment, but levels similar to those of the endogenous protein and correct trafficking were only reached by 480 or 180 min, respectively (fig. S1, A and B).

Plaque assays reflect multiple lytic cycles, including invasion, replication, egress, and invasion of neighboring cells. Parasites expressing ddROM4 or yellow fluorescent protein (RH-2YFP) formed plaques of similar size with or without Shld-1, indicating that overexpression of ROM4 was not detrimental. The ddROM1_{S-A} parasites formed slightly smaller plaques in the presence of Shld-1, suggesting a modest growth defect. The ddROM4_{S-A} parasites produced no plaques in the presence of Shld-1 (Fig. 1C), indicating that its expression exerts a dominant negative effect. Closer examination of the ddROM4_{S-A} phenotype revealed an unanticipated defect in replication. Quantification of the number of parasites per vacuole 24 hours postinvasion indicated that the RH-2YFP and ddROM1_{S-A} parasites replicated at a similar rate regardless of the presence of Shld-1, whereas the ddROM4 parasites grew slightly better. In contrast, the ddROM4_{S-A} line grew normally without Shld-1 but was severely impaired in the presence of Shld-1 (Fig. 1D and fig. S1C). Modification of the extreme C terminus of rhomboids

interferes with activity (13, 14, 16), and inclusion of a C-terminal Ty-1 epitope tag abrogated the deleterious effect of ddROM4_{S-A} (fig. S2), supporting the notion that stabilization of ddROM4_{S-A} produces a dominant-negative effect.

Delivery of organelles into daughter cells during replication occurs in a highly coordinated fashion, starting with the centriole and Golgi, followed by the apicoplast, the nucleus, and endoplasmic reticulum, and finishing with the mitochondrion and de novo synthesis of the micronemes and rhoptries (21). To define the point of cell-cycle arrest after ddROM4_{S-A} stabilization, we scrutinized the integrity and inheritance of various organelles. Though the inner membrane complex and apicoplast appeared normal, the mitochondrion, micronemes, rhoptries, and nuclei were defective in arrested parasites (Fig. 2, fig. S3, and tables S2 and S3) in a phenotype characteristic of an arrest late in the cell cycle at the S phase (22). ddROM4_{S-A} stabilization also reduced the amount of vacuoles containing developing daughter cells (Fig. 2B and table S2).

Because ROM4 plays an important though nonessential role during invasion (15), we investigated the effect of ddROM4_{S-A} stabilization on motility and invasion. As prolonged Shld-1 treatment of intracellular parasites prevented egress as a direct result of the block in replication, we stabilized ddROM4_{S-A} in nondividing extracellular parasites. Consistent with (15), there was a modest defect in invasion and gliding (fig. S4).

To determine whether the impairment in cell division after ddROM4_{S-A} stabilization was dependent on an invasion-related event, we performed pulse-chase assays. Although ddROM4_{S-A} parasites treated with Shld-1 for 12 hours before egress and for 6 hours postinvasion recovered and underwent normal cell division (Fig. 3A), parasites treated at 6 hours postinvasion were impaired (Fig. 3B). Thus, the defect is reversible and independent of invasion, resulting from the non-cleavage of one or more substrates at the intracellular parasite surface, whose processing is required to trigger or maintain replication.

All *T. gondii* rhomboid substrates characterized to date are micronemal proteins. Among these, AMA1 is unique in that it functions exclusively during invasion (2), it is detected on invading parasites (3), and its C-terminal tail is detectable in the newly invaded (6, 7, 9) parasites. We hypothesized that the AMA1 cytosolic tail generated by ROM4 cleavage during invasion triggers parasite replication and that stimulation of each replicative cycle is engendered by further cleavage of AMA1 or another substrate. To test this hypothesis, we determined whether the block in division could be reverted by expressing the *T. gondii* (ddAMA1) or *P. falciparum* (ddPfAMA1) AMA1 tail in either the WT form or mutated in residues important for the AMA1 invasion-related function (23–25). We substituted with Ala either the conserved Phe and Trp (23) (FW) (26) residues in TgAMA1 (ddAMA1_{FW-AA}) or the Ser residue phosphorylated in PfAMA1 (25)

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(ddPfAMA1_{S-A}) (Fig. 4A). Expression of all transgenes was Shld-1-regulated (fig. S5) and did not affect the growth of WT or *ddROM4* parasites (fig. S6), but it was able to rescue the replication phenotype of *ddROM4*_{S-A} parasites (Fig. 4, B and C, and fig. S7), indicating independent, dual functions for AMA1 in replication and invasion. To identify residues involved in the replication function of AMA1, we tested

ddAMA1 constructs carrying an Ala replacement at the conserved C-terminal Tyr residue (ddAMA1_{DY-AA}) or with the most N-terminal region (ddAMA1₅₃₅₋₅₇₀) or the 20 most C-terminal residues (ddAMA1₅₀₄₋₅₄₉) deleted (Fig. 4A). None of the mutants impaired function (Fig. 4, B and C, and fig. S7), suggesting that the conserved central region PSDLMQEAEP is important for function. To assess the specificity of these

results, we expressed the MIC2 cleavage product (ddMIC2) and verified that it did not affect parasite growth (fig. S6) or complement the *ddROM4*_{S-A}-mediated arrest (Fig. 4, B and C).

Two previous studies found no evidence for a role of ROM4 or AMA1 in replication (2, 15). Reexamination of the phenotype of parasites conditionally depleted for *AMA1* (2) found that they were modestly affected in division (fig. S8), con-

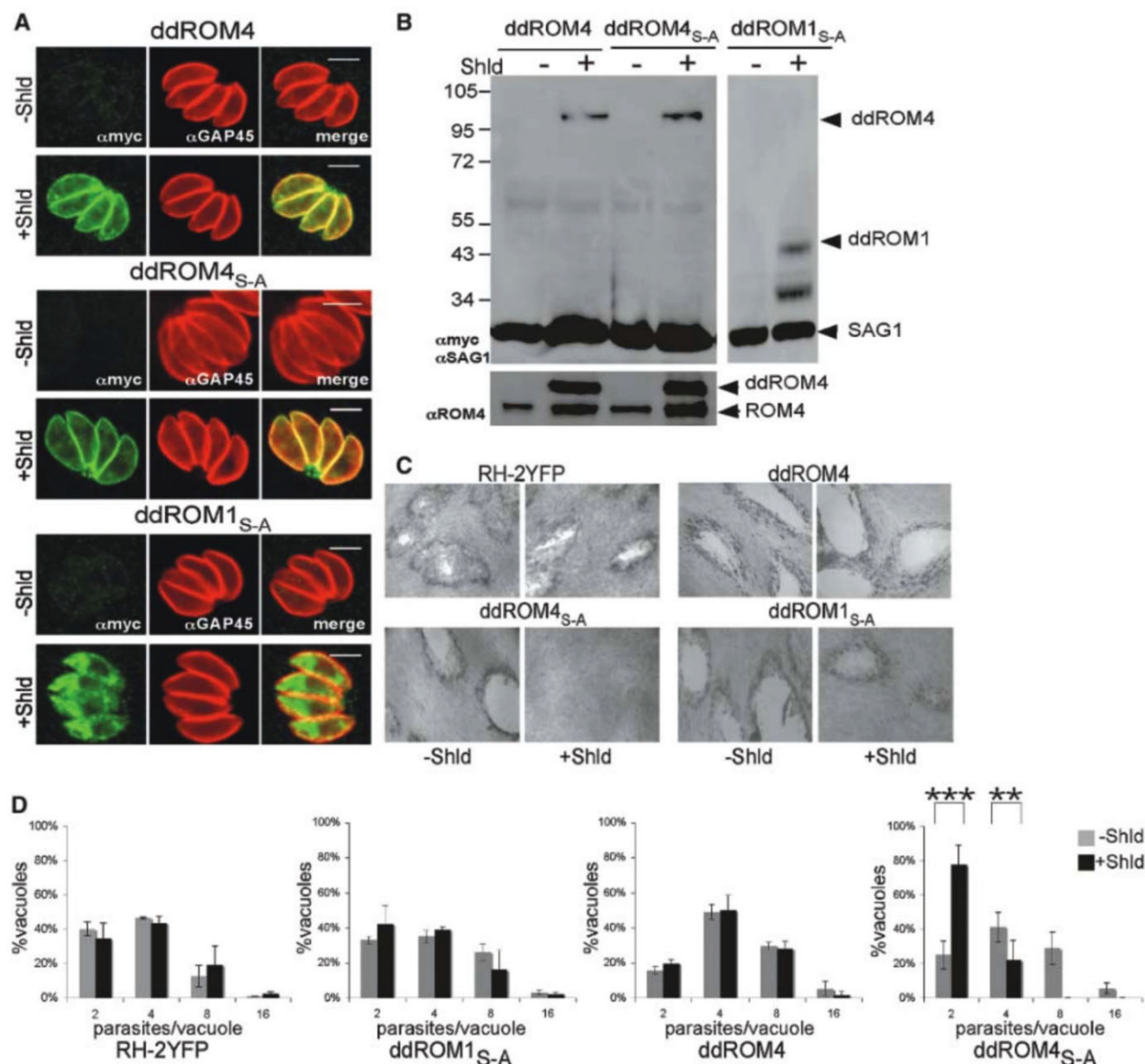


Fig. 1. Expression of *ddROM4*_{S-A} severely impairs intracellular growth. Stabilization of *ddROM4*, *ddROM4*_{S-A}, and *ddROM1*_{S-A} expression on parasites treated 12 hours $\pm$ Shld-1, as shown by indirect immunofluorescence assay [(A), green] or Western-blot [(B), top panels] with α -myc antibodies. The additional lower form of *ddROM1*_{S-A} (B) probably corresponds to a degradation product or otherwise modified form (13). Gliding-associated protein 45 (α -GAP45) labels the inner-membrane complex [(A), red]. Scale bars in (A): 5 μ m. ROM4 N-terminal (α -ROM4) antibodies were used to compare the expression level of *ddROM4*/*ddROM4*_{S-A} to endogenous ROM4 [(B), bottom panel]. The surface marker surface antigen 1 (α -SAG1)

served as loading control [(B), top panels]. (C) Plaque assays of *RH-2YFP*, *ddROM4*, *ddROM4*_{S-A}, and *ddROM1*_{S-A} parasites grown 7 days $\pm$ Shld-1. The assays were performed simultaneously for the same parasite strain $\pm$ Shld-1. (D) Replication profile of *RH-2YFP*, *ddROM1*_{S-A}, *ddROM4*, and *ddROM4*_{S-A} parasites pretreated 12 hours $\pm$ Shld-1 before host cell egress and for the time of the assay in a total of 36 hours. We counted the number of parasites per vacuole 24 hours after host cell inoculation. Asterisks indicate statistically significant results (** P = 0.004; *** P = 0.0005), as determined by the Student's t test. Data are represented as mean $\pm$ SD (error bars) of four independent experiments.

Fig. 2. *ddROM4_{S-A}* parasites arrest late in the cell cycle. Indirect immunofluorescence assay of *ddROM4_{S-A}* parasites after 24 hours $\pm$ Shld-1 showing the mitochondrion (α -F1 adenosine triphosphatase β subunit, green) broken down (A); replication arrested after a single round of division, as determined by staining of the nascent apical cones of the mother and daughter parasites (α -ISP1, green) only in nontreated parasites [(B), arrowheads]; and defective karyocytokinesis with the nuclei enlarged and uncondensed [(C), arrowheads]. In red, parasites are labeled with α -GAP45. Longitudinal section through two daughters cells after 18 hours plus (D) or minus (E) Shld-1 and after 36 hours plus Shld-1 (F). (G) Section through a nontreated vacuole at 36 hours showing three cycles of endodyogeny. (H) Detail of a vacuole 18 hours plus Shld-1, showing the mitochondrion running between the posterior pole and the residual body, which is left after the budding of daughter cells from the mother parasite. (I) Longitudinal section through one of two daughters at 18 hours plus Shld-1, showing the elongated and lobed appearance of the nucleus. N, nucleus; DG, dense granule; R, rhoptry; C, conoid; MN, micronemes; RB, residual body; Mi, mitochondrion; PP, posterior pole. Scale bars: (A) to (C), 5 μ m; (D) to (G), 1 μ m; (H) and (I), 0.5 μ m.

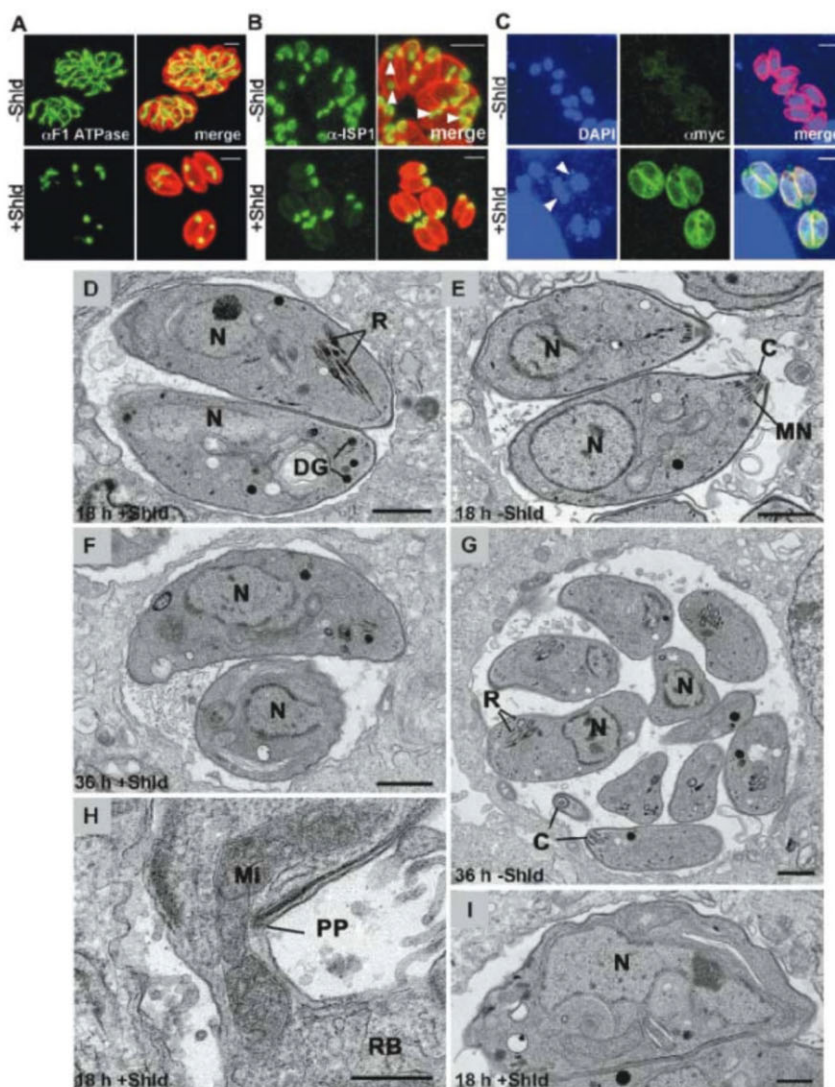


Fig. 3. The dominant negative effect of *ddROM4_{S-A}* is reversible and independent of invasion. Intracellular *ddROM4* and *ddROM4_{S-A}* parasites were treated 12 hours with Shld-1 before host cell egress and Shld-1 was then removed 6 hours postinvasion [(A), blue bars], or parasites were allowed to invade and Shld-1 was added 6 hours postinvasion and was maintained for the duration of the assay in a total of 18 hours [(B), blue bars]. Replication was compared to either nontreated parasites (gray bars) or parasites pretreated 12 hours with Shld-1 before host cell egress and for the time of the assay in a total of 36 hours (black bars). We counted the number of parasites per vacuole 24 hours after invasion. Asterisks indicate statistically significant results (* P = 0.01; ** P = 0.004), as determined with the Student's t test. Data are represented as mean $\pm$ SD (error bars) of four independent experiments.

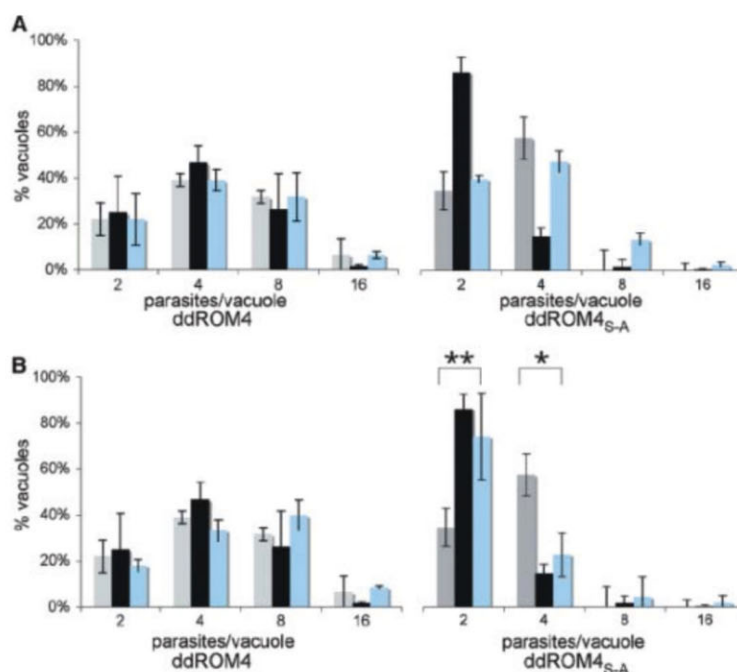
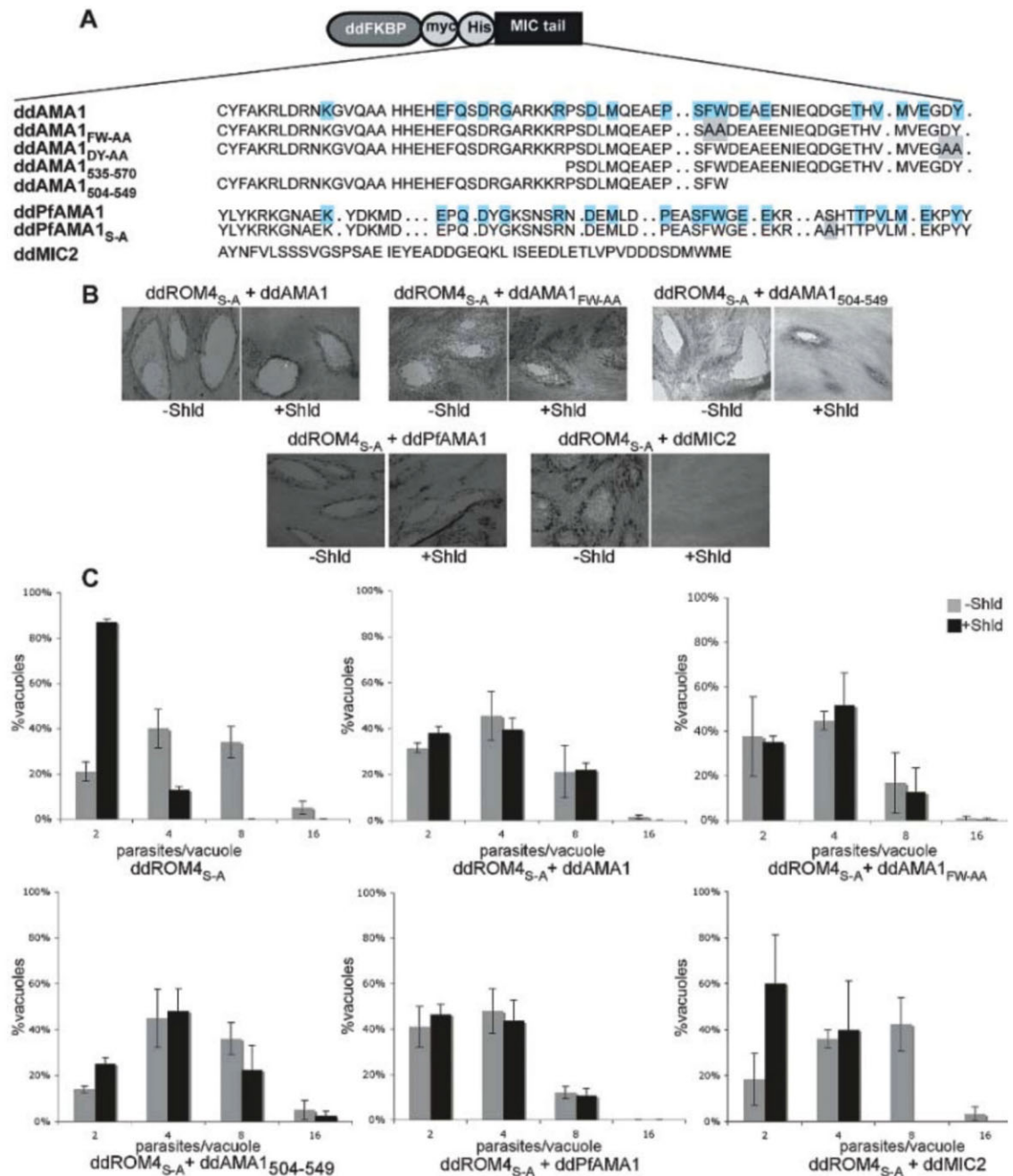


Fig. 4. Expression of ddAMA1, ddAMA1_{FW-AA}, and ddPfAMA1 trans-complements the negative effect of ddROM4_{S-A} on replication. (A) Schematic of the *T. gondii* (ddAMA1, ddAMA1_{FW-AA}, ddAMA1_{DY-AA}, ddAMA1₅₃₅₋₅₇₀, ddAMA1₅₀₄₋₅₄₉ and ddMIC2) and *P. falciparum* (ddPfAMA1 and ddPfAMA1_{S-A}) fusion proteins used in this study. The sequence of the cloned tails is shown. Mutated residues are boxed in gray; residues conserved in the *T. gondii* and *P. falciparum* AMA1 tail are boxed in blue. (B) Plaque assays of ddROM4_{S-A} parasites expressing ddAMA1, ddAMA1_{FW-AA}, ddAMA1₅₀₄₋₅₄₉, ddPfAMA1, or ddMIC2. Parasites were incubated 7 days $\pm$ Shld-1 before fixation and Giemsa staining. The assays were only performed simultaneously for the same parasite strain $\pm$ Shld-1. (C) Intracellular replication assays of parasites expressing ddROM4_{S-A} alone or in combination with ddAMA1, ddAMA1_{FW-AA}, ddMIC2, ddAMA1₅₀₄₋₅₄₉, or ddPfAMA1. Parasites were treated 24 hours $\pm$ Shld-1 before fixation. Data are represented as mean $\pm$ SD (error bars) of three independent experiments.



firming a role for AMA1 in replication. Failure to observe a replication defect in (15) may have been the result of an inherent limitation of the system [see supporting online material (SOM)].

The apicomplexan life cycle consists of consecutive transmissive and replicative phases. Premature differentiation into replicative forms would be potentially lethal, so commitment to cell division needs to be tightly regulated in time and space. We show here that a mechanism of regulated intramembrane proteolysis (27) acting on AMA1 is implicated in a signaling pathway leading to replication (see SOM). Our study highlights a role in this process for one of the most conserved apicomplexan proteins and shows that this group of parasites has opted to use invasion

molecules to couple invasion with replicative growth.

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times. As predicted, infants attended longer to the Unexpected Outcome, in which a big agent bowed and yielded to a small one [mean (M) = 20.0 ± 4.2 (SEM)], than vice versa [$M = 12.0 \pm 3.2$ (SEM); $F_{(1,14)} = 9.4$, $P = 0.008$; partial ratio of variance accounted for (η^2) = 0.40; table S1] (31).

A second experiment explored the developmental course of coming to expect that a small, novel agent will yield to a larger one in their first right-of-way conflict, using stimuli identical to those in experiment 1. Participants were 8-month-old ($n = 16$), 9-month-old ($n = 16$), 10-month-old ($n = 16$), and 12- to 13-month-old ($n = 16$) infants (31).

A repeated-measures ANOVA with test trials (expected or unexpected) varied within subjects and presentation order (expected first or unexpected first) and age category (8, 9, 10, and 12 to 13 months) varied between subjects again revealed that infants' attention was drawn especially to those trials in which the larger agent made way for the smaller one [$M = 17.2 \pm 1.6$ (SEM)], rather than the reverse [$M = 11.8 \pm 1.2$ (SEM); $F_{(1, 56)} = 11.40$, $P = 0.001$, partial $\eta^2 = 0.17$]. This effect interacted with participant age ($F_{(3, 56)} = 3.14$, $P = 0.032$, partial $\eta^2 = 0.14$). Planned follow-up analyses (with one-tailed tests of the unidirectional hypothesis that the main effects of experiments 1 and 2 should replicate within each age group of the present experiment) demonstrated that 8-month-olds failed [$M_{\text{BigYields}} = 6.0 \pm 1.0$ (SEM), $M_{\text{SmallYields}} = 8.2 \pm 2.5$ (SEM); paired-samples $t_{(15)} = -1.15$, $P_{(\text{one-tailed})} = 0.13$], and 9-month-olds marginally succeeded [$M_{\text{BigYields}} = 11.9 \pm 3.0$ (SEM), $M_{\text{SmallYields}} = 6.7 \pm 1.9$ (SEM), paired-samples $t_{(15)} = 1.54$, $P_{(\text{one-tailed})} = 0.073$], in differentiating between unexpected and expected test trials, whereas 10-month-olds [$M_{\text{BigYields}} = 21.7 \pm 4.1$ (SEM), $M_{\text{SmallYields}} = 14.4 \pm 2.6$ (SEM); paired-samples $t_{(15)} = 1.78$, $P_{(\text{one-tailed})} = 0.048$] and 12- to 13-month-olds [$M_{\text{BigYields}} = 29.0 \pm 3.9$ (SEM), $M_{\text{SmallYields}} = 18.0 \pm 2.9$ (SEM), paired-samples $t_{(15)} = 3.49$, $P_{(\text{one-tailed})} = 0.0015$] were able to do so (Fig. 1 and table S1) (31).

Replicating the results of experiment 1, which used children 11 months and older, experiment 2 thus demonstrates that the difference of attention when a large agent bows and yields to a smaller agent rather than vice versa develops between 8 and 10 months.

It is possible that the results of experiments 1 and 2 reflect merely that there is more mass in motion, and thus perhaps more salient motion catching the infants' attention, when the larger rather than the smaller agent bows and scoots away for the other. As an initial control for this possibility, a third Isolated Motion Control experiment (fig. S2) exposed 11- to 16-month-olds ($n = 16$) to the same familiarization events used in experiments 1 and 2 (movies S1 and S2), but without conflicting goals in intertrials and test trials. Instead, either the large or small agent was alone on stage, performing the identical motions as in the Conflicting Goal trials. Two intertrials (movies S6 and S7) showed infants the actions of each agent immedi-

ately before it bowed and scooted back on subsequent test trials (movies S8 and S9), while not implying conflicting goals, because each agent was alone on the stage (31). Hence, if the results of the Conflicting Goals experiments had been driven by differences in the total mass in motion across the unexpected and expected test trials, then infants should also differentiate the Isolated Motion test trials. Conversely, if the results so far reflect infants' use of relative size as a cue for dominance rank predicting the outcome of a conflict of goals, then infants should not differentiate the current Isolated Motion trials.

Here, a repeated-measures ANOVA with the test factor (expected or unexpected) varied within subjects and presentation order varied between subjects revealed that 11- to 16-month-olds no longer differentiated between a large [$M = 6.9 \pm 2.2$ (SEM)] and small [$M = 6.3 \pm 2.0$ (SEM)] agent bowing and scooting away [$F_{(1,14)} = 0.089$, ns (not significant); table S1] (31). Pooling these data with those of experiment 1, the 11- to 16-month-olds in these two experiments looked

equally long at the identical familiarization events, ruling out preexisting group differences in attentiveness. Crucially, an interaction confirmed that infants' differentiation of unexpected and expected test trials varied significantly across experiments in such a way that the size of the bowing agent mattered in the Conflicting Goals experiment but not in the Isolated Motion Control experiment (Test X Experiment: $F_{(1,28)} = 4.75$, $P = 0.038$, partial $\eta^2 = 0.15$) (Fig. 2) (31).

Still, it is possible that infants are only sensitive to the greater mass in motion when the large block prostrates itself if cues to relative size are also present. This was not the case in the Isolated Motion Control experiment, because infants never watched the two agents together. Thus, low-level perceptual factors might still have driven the results presented so far. Experiment 4 (Motion Behind Control, fig. S3) addressed this possibility and explored another alternative interpretation of the results of experiments 1 and 2: that young infants merely expect that smaller agents are more likely to fall over than are bigger ones.

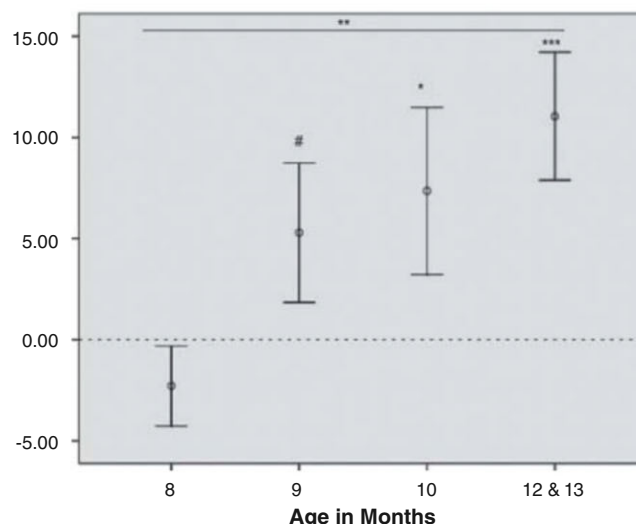


Fig. 1. Developmental onset of the Conflicting Goals effect (experiment 2). Continued mean differential looking times ($\pm$ SEM) at unexpected over expected test trials by categorical age, once the animations had frozen to stills (after 19.1 s), are shown. $N = 64$ infant participants (8-, 9-, 10-, and 12- to 13-month-olds). # $P_{(\text{one-tailed})} = 0.073$, * $P_{(\text{one-tailed})} = 0.048$, ** $P = 0.032$, *** $P_{(\text{one-tailed})} = 0.0015$.

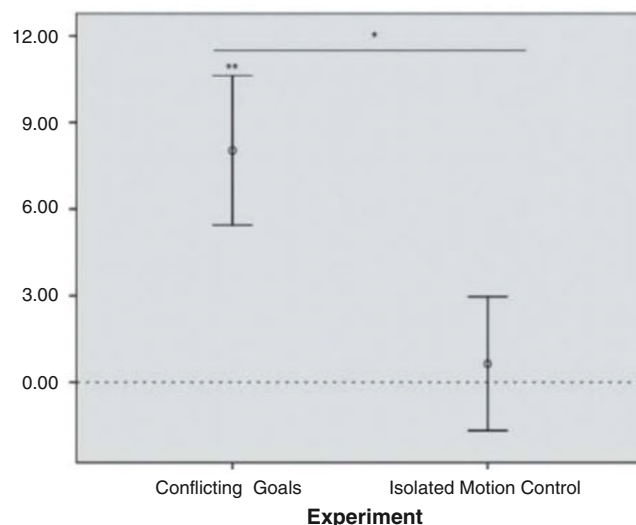


Fig. 2. Conflicting Goals (experiment 1) versus Isolated Motion Control (experiment 3). Continued mean differential looking times ($\pm$ SEM) at unexpected over expected test trials by experimental condition, once the animations had frozen to stills (after 19.1 and 13.2 s, respectively), are shown. $N = 32$ infant participants (11- to 16-month-olds). * $P < 0.05$, ** $P_{(\text{one-tailed})} < 0.01$.

context (Fig. 4) (31). This rules out the possibility that the greater complexity of the occlusion events in the unexpected Conflicting Goals test trials drove the infants' interest to them.

Together, the five experiments presented here show that preverbal infants use relative size to predict which of two novel agents has the right of way. This effect is not driven by increased saliency of the greater area in motion when the large agent bows; nor by expectations that small, rather than large, agents or objects fall over; nor by differential attention to partial versus full occlusion when the agents pass each other. In addition to the results from the three quite different control conditions, the fact that 8-month-olds fail, 9-month-olds marginally succeed, and 10- and 12- to 13-month-olds robustly succeed at differentiating the Conflicting Goals outcomes speaks further against low-level perceptual saliency accounts that would predict no such age differences. One final detail of our data provides additional support for the interpretation that our findings in experiments 1 and 2 depend on the representations of dominance ranks for predicting the outcomes of conflicting goals. What the qualitatively different control experiments have in common is that they do not establish conflicting goals, and infants were far less interested in all of them than in the Conflicting Goals test trials (31).

Other lean interpretations that make no reference to goal-directed agents and social dominance cannot account for our data. The animations were designed so that they would not invoke mechanistic physics. One agent did not knock over the other one; instead the two agents moved back from each other and paused, before one of them lay down forwardly, opposite to what billiard-ball causality would entail. Finally, a lean account might argue that infants' attention could simply be drawn to relative (nonsocial) size difference. However, relative size difference is maximized in the expected Conflicting Goals test trials (fig. S1 and movies S4 and S5), whereas we found that infants attend more to the unexpected trials, and infants did not differentiate the exact same relative size differences in the Motion Behind test trials (fig. S3 and movies S16 and S17).

Social relations are irreducible to features of individual agents. Although a social interaction gives evidence that intentional agents are present, the presence of several intentional agents alone does not predict the kind of social relationship they have with one another. Previous research has documented that young infants represent interactions among others in terms of affiliative and cooperative relations (13–16). The present studies show that preverbal infants also represent dominance between competing agents. Moreover, consistent with existing research on infants' representations of intentional agency (7–12), our evidence suggests that this conceptual understanding develops between 8 and 10 months of age.

Nine- and 10-month-old infants are too young to have actively participated in dominance

fight, and American infants are unlikely to have watched small agents bow and prostrate in subordination to others of more formidable physical size, such as their parents. They may have experienced older siblings taking their toys or observed older siblings struggle with each other, and learned that the bigger one often gets his or her way over the little one. If infants use these experiences to predict what will happen in our animations, they must see the similarity between previous experiences and the present animations, even though the spatiotemporal parameters of the agents and their physical movements do not exactly match those they have experienced. Hence, representations of conflicting goals and social dominance, rather than spatiotemporal primitives, must underpin the relevant similarity metric that allows infants to apply their experiences to the situations depicted in our animations.

We cannot know, at present, what aspects of our stimuli led the children to encode the familiarization and intertrial events in experiments 1 and 2 in terms of conflicting goals between two agents. It is likely that the presence of face-like features contributed to infants' categorization of the blue and green blocks as agents (13, 14). But equifinality of motion is also sufficient to establish goals for novel agents with no facial features (9). It is also uncertain whether infants would have extrapolated that the agents' goals conflict had the agents not bumped into each other when physically blocking each other's way (indeed, the infants might have thought that the agents could pass or jump over each other so that both could complete their paths to the end of the stage). These results also leave open just what about the unexpected conflicting goals event is unexpected. Infants' attention may be drawn when the smaller agent prevails, or when the larger agent displays features of subordination (by prostrating itself), or both. Importantly, our results do not yet address which other conflicting goals and features of dominance infants can represent, nor whether infants expect the dominance relation between two agents to be stable across different contexts of conflicting goals, even when the physical dominance cue of relative size is absent.

A crucial task for the developing child is to learn the social structure of his or her world, in order to interact appropriately with kin and non-kin, friend and foe, superiors, inferiors, and peers. Constraints on infants' mental representations of social relations may direct infants' attention to the relevant features among the myriad stimuli present in any social scenario and assist them in interpreting social interactions (3–6). Our finding that preverbal infants mentally represent conflicting goals and social dominance between two agents suggests that just as infants possess early-developing mechanisms for learning about the physical world and the world of individual intentional agents (3), they also have early-developing representational resources tailored to understanding the social world, allowing infants to under-

stand and learn the dominance structures that surround them.

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31. See supporting material on Science Online.
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Supporting Online Material

www.sciencemag.org/cgi/content/full/331/6016/477/DC1

Materials and Methods

SOM Text

Figs. S1 to S4

Table S1

Reference

Movies S1 to S25

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Science Careers

From the journal *Science*

POSITIONS OPEN

TENURE-TRACK FACULTY POSITION in Teaching Biochemistry

The Department of Biochemistry and Molecular Biology at The George Washington University Medical Center invites applications for a tenure-track ASSISTANT/ASSOCIATE PROFESSOR beginning in September 2011.

Basic qualifications: Applicants must have a Ph.D. degree in the life sciences (preferably in biochemistry) and have had extensive experience teaching biochemistry to medical students as well as to undergraduate and graduate (M.S.) students.

Preferred qualifications: Preference will be given to candidates with experience in teaching biochemistry and genetics to medical students involving didactic lectures, problem-based learning, and small group discussions. Candidates with this experience as well as training and interests in a medically relevant field are preferred. Strong communication and collaborative skills and a commitment to innovative approaches to education are also desirable.

Application process: Interested applicants must send complete curriculum vitae; a statement of teaching experience (including evidence of teaching excellence), philosophy, and interests; and three formal letters of recommendation to: **Ms. Kelly D. Crist, Executive Coordinator, Department of Biochemistry and Molecular Biology, Faculty Search, The George Washington University Medical Center, Suite 530, 2300 Eye St NW, Washington, DC 20037.** Or, electronically to e-mail: bcmkdc@gwumc.edu.

Review of applications by the Search Committee will begin on March 15, 2011 and will continue until the position has been filled. Only complete applications will be considered.

The George Washington University is an Affirmative Action/Equal Opportunity Employer.

COURSE ANNOUNCEMENT Environmental Biomechanics, Physiology, and Genomics of Marine Species

Marine species are widely used as a model system in community ecology, physiology, and genetics. Many of the factors responsible for structuring these communities are abiotic variables such as wave exposure, temperature, wind speed, and light. The physical and biological environment also sets the geographic scale for dispersal, adaptation, and gene flow. The interaction between the physical environment and individual fitness or performance is especially topical in light of climate change. This four-week summer course is designed to offer experimental ecologists and physiologists theoretical and hands-on instruction in cutting-edge methods in biomechanics, physiological molecular biology, and genome-wide investigations of population differentiation and adaptation. The relationships between complex environmental mosaics and genome responses in the context of future climate change will be a major theme.

Instructors: **Drs. Mark Denny, Steve Palumbi and George Somero.** Dates: June 13 through July 8, 2011. Independent research following the course is possible. Location: **Hopkins Marine Station, Stanford University, Pacific Grove, CA 93950-3094.** For additional information, including a course prospectus and instructions for application, see website: <http://hms.stanford.edu/HMSweb/mech.htm> or contact the instructors (e-mails: mwdenny@stanford.edu, spalumbi@stanford.edu, or somero@stanford.edu). Deadline for receipt of all application materials is April 1, 2011.

HARVARD MEDICAL SCHOOL

POSTDOCTORAL POSITION available to study the molecular and cellular mechanisms of Alzheimer's and Parkinson's diseases (*Nature* 460:632, 2009; *PNAS* 107:9879, 2010; *J. Neurosci.* 30:13066, 2010; *Science* 330:1055, 2010). Recent Ph.D.s with strong background in molecular biology, biochemistry, or electrophysiology are encouraged to apply. Prior experience in slice physiology, calcium imaging, or mitochondrial analysis is preferred. Send curriculum vitae to **Dr. Jie Shen** (website: <http://www.shenlab.net>) at e-mail: jshen@rics.bwh.harvard.edu.

POSITIONS OPEN

RADIOCHEMISTRY FACULTY POSITION

The University of Nevada, Las Vegas (UNLV), Department of Chemistry, invites applications for a tenure-track ASSISTANT, ASSOCIATE, or FULL PROFESSOR with emphasis on Radiochemistry. The UNLV Radiochemistry program (website: <http://radchem.nevada.edu>) has a research focus on the chemistry of technetium and the actinides in solids, solutions, the environment, and chemical syntheses. Applicants must hold a Doctorate in chemistry, radiochemistry, or a closely related field. Applicants should have demonstrated records of scholarly productivity and a track record in external funding, commensurate with the position level. The position requires teaching undergraduate and graduate-level courses in both chemistry and radiochemistry. In addition, mentoring graduate and undergraduate students in an internationally competitive research environment is required. The successful applicant is expected to provide leadership in the UNLV Radiochemistry program and the UNLV Chemistry Department, commensurate with the position level (Assistant, Associate, or Full Professor), utilize and enhance existing facilities and laboratory equipment in the UNLV Radiochemistry Program, interact closely with other research and academic faculty in the program, and maintain a rigorous, externally funded research program. For a complete position description and application details, please visit website: <http://jobs.unlv.edu> or call telephone: 702-895-2894 for assistance. *Equal Employment Opportunity/Affirmative Action Employer.*

POSTDOCTORAL FELLOWSHIPS Cedars-Sinai Medical Center

Applications are solicited for federally funded basic, clinical, and translational medicine research fellowships in Endocrinology, Diabetes, and Metabolism at Cedars-Sinai Medical Center.

We are an academic medical center and major teaching hospital of the David Geffen School of Medicine at UCLA. We seek qualified applicants with M.D., Ph.D., D.V.M., or D.D.S. degrees who seek postdoctoral training from a strong faculty of research scientists.

Applicants must be U.S. citizens or possess permanent residence status.

Underrepresented minorities and women are particularly encouraged to apply, specifically African Americans, Hispanic Americans, Native Americans, Alaskan Natives, and Pacific Islanders.

Submit inquiries to:

**Office of Academic Affairs
Cedars-Sinai Medical Center
8700 Beverly Boulevard
Los Angeles, CA 90048
E-mail: billy.gellepis@cshs.org
Website: <http://www.csmc.edu>**

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The Medical Faculty at the Goethe-University Frankfurt in cooperation with the Foundation "Institute for Biomedical Research - Georg-Speyer-Haus - (GSH)" invites applications for the following position:

Full Professorship (W3) in Cancer Biology (Succession Prof. Bernd Groner, PhD)

The holder of the position will be Director of the Georg-Speyer-Haus in Frankfurt am Main. The Georg-Speyer-Haus is an academic research institute with a great tradition in biomedical research, located adjacent to the campus of the University Clinics of the Goethe-University in Frankfurt am Main. The Institute is seeking an exceptional candidate to lead the scientific activities of the Georg-Speyer-Haus, to supervise its organization and to serve as a member of the Medical Faculty of the Goethe-University, Frankfurt.

The Georg-Speyer-Haus is an independent research foundation and employs a staff of about 100 members, currently organized into 9 research groups. The research program is focussed on cancer biology and molecular and cellular therapy, and comprises basic and translational research in the areas of solid tumors as well as hematological malignancies and inherited diseases. The individual research groups are working on aspects of stem cell biology and stem cell therapy, cells of the immune system in cancer therapy and immunodeficiency, and signal transduction in cancer cells. The Georg-Speyer-Haus is supported by the State of Hessen and the Federal Ministry of Health with a stable significant budget complemented by competitive research grants from public and private funding organizations.

The position provides a unique opportunity to lead an institute dedicated to uncovering new knowledge in the area of cancer biology and the exploitation of these insights for clinical applications through innovative technologies in close cooperation with the Medical Faculty of the Goethe-University. The candidate must be committed to scientific excellence, be able to integrate collaborative approaches across basic and clinical disciplines, design and implement successful research strategies and lead a research group within the institute. A high degree of dedication and experience in academic teaching are explicitly expected, especially in the planned course in Molecular Medicine.

Candidates for this position must have an MD or a PhD degree, an internationally recognized reputation in basic and applied cancer research, senior level research leadership experience and the ability to manage complex research programs and budgets. In addition, superb communicative and organizational skills are important prerequisites.

The designated salary for the position is based on "W3" on the German university scale or equivalent. Frankfurt University is an equal opportunity employer.

Applications from or nominations of female candidates are encouraged. For further information regarding the general conditions for professorial appointments, please see: <http://www.uni-frankfurt.de/aktuelles/ausschreibung/professuren/index.html>

To be considered for this position, please submit a curriculum vitae, a bibliography, a short description of the major achievements, a list of current funding and a scientific concept for future research in the institute. Applications should be sent within **four weeks** after publication of this advertisement to: **The Dean, Faculty of Medicine of the Johann Wolfgang Goethe-University, Theodor-Stern-Kai 7, D-60590 Frankfurt/Main**, E-Mail: dekan@kgu.de and (in electronic form) to: **Dr. Rolf E. Breuer, Chairman of the Board of the Foundation "Georg-Speyer-Haus", c/o Deutsche Bank, Taunusanlage 17, D-60325 Frankfurt am Main**, E-Mail: claudia.rode@db.com



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Northwest A&F University

NORTHWEST A&F UNIVERSITY FACULTY POSITIONS in MULTIPLE DISCIPLINES

The Northwest A&F University (<http://www.nwsuaf.edu.cn>) invites applications for full-time faculty positions in the following areas: biological sciences either cellular biology, molecular biology, ecology, microbiology, physiology, entomology or plant pathology; agricultural sciences either soil sciences, crop sciences, food sciences, animal science or veterinary medicine; engineering either mechanical engineering or computer engineering; and social sciences either agricultural and resource economics or sociology, etc. The successful candidates will have a doctoral degree or equivalent degree and experience in academic research and teaching. Outstanding applicants are encouraged to apply.

The successful candidates will be provided with internationally competitive startup support, salary, benefits and housing (apartments) according to qualifications and experience.

Applicants should submit: (1) letter describing teaching and research experiences and career goals, (2) curriculum vitae, and (3) three letters of reference directly from the referees to: Talent Introduction Office, Personnel Division, Northwest A&F University, 3 Taicheng Rd., Yangling, Shaanxi 712100, P.R.China. Formal inquiries can be directed to Mr Zhang Pengfei (or Ms Chen Xiaoyan): E-mail: rencaike@nwsuaf.edu.cn or rencaiban@gmail.com; Telephone: + 86-29-87082855. Interested persons can also visit our website: <http://rcb.nwsuaf.edu.cn/>. Consideration of applications will start immediately and the positions will remain open until suitable candidates are selected.



Flow Cytometry Core Director

The University of Connecticut Health Center seeks candidates at the Ph.D. level with extensive experience with flow cytometry instrumentation and applications to direct a heavily utilized and well equipped flow cytometry core (<http://flowcytometry.uchc.edu/>). The facility currently houses a B-D FACSVantage SE, a FACSARIA, three LSRII and two FACSCaliburs. The director will be an expert in operation of the equipment and will be expected to direct the daily operations of the facility. He/she will independently design and implement new cytometry protocols and assist users with specialized applications. A faculty appointment is associated with this position.

Salary will be commensurate with experience. UCHC offers an excellent benefits package. Interested applicants should apply at <https://jobs.uchc.edu>, search number 2011-622 and upload a curriculum vitae and the names of three references through the website. Questions regarding this search should be addressed to Leo Lefrancois at llefranc@neuron.uchc.edu

*UCHC is an Equal Opportunity Employer
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The Mount Sinai School of Medicine is internationally acclaimed for excellence in clinical care, education, and scientific research.

CHAIR, DEPARTMENT OF GENETICS AND GENOMIC SCIENCES

Mount Sinai School of Medicine, New York, NY

We seek a national and international leader to serve as Chair of this outstanding academic department. Candidates must have an MD and/or PhD, a superior record of scientific achievement in genetics or genomics research, demonstrated commitment to education, and proven leadership ability. The Chair will have a unique opportunity to build upon an already leading department.

Mount Sinai School of Medicine and Mount Sinai Hospital are among the world's leading biomedical institutions. The Medical Center is in the middle of a \$1 billion capital campaign in support of our \$2.25 billion strategic plan whose primary focus is on basic and translational research leading to therapeutic discoveries and on the delivery of outstanding clinical care. A major new research building, the Center for Science and Medicine, focusing on translational research is under construction with an expected occupancy mid-2012. Our leadership is committed to maintaining and growing this outstanding Genetics and Genomic Sciences department. The Medical Center will provide the exceptional resources necessary to recruit the highest quality scientists.

We offer a competitive salary and an excellent benefits package. Please send a letter of application or nomination, with curriculum vitae, to: **Dr. Eric J. Nestler, Director, The Friedman Brain Institute, Mount Sinai School of Medicine, One Gustave Levy Place, Box 1065, New York, NY 10029.**

To receive full consideration, applications should arrive by March 11, 2011.

Mount Sinai Medical Center is an equal opportunity/affirmative action employer. We recognize the power and importance of a diverse employee population and strongly encourage applicants with various experiences and backgrounds.

Mount Sinai Medical Center--An EEO/AA-D/V Employer



**Postdoctoral Positions Available
Program in Genomics of Differentiation
NATIONAL INSTITUTE OF CHILD HEALTH AND HUMAN DEVELOPMENT**



Fully funded postdoctoral positions are available in the NICHD Program in Genomics of Differentiation (PGD). The PGD is a diverse and highly interactive program in basic cellular, molecular, and developmental biology research at the National Institutes of Health in Bethesda, Maryland, just outside of Washington, DC. The 19 laboratories making up the PGD encompass a wide variety of research areas, with strong emphasis on developmental patterning and differentiation, chromatin dynamics and epigenetics, the immune system, the viral life cycle, DNA replication, gene regulation, and RNA metabolism. Program investigators perform research using a wide variety of models including viruses, bacteria, mammalian cell culture, yeast, fruit flies, zebrafish, frogs, and mice. Environment, resources, and stipend support are excellent- for additional information on the PGD and its researchers see <http://science.nichd.nih.gov/confluence/display/pgd/Home>. Interested applicants with a Ph.D. or M.D. and less than 5 years' postdoctoral experience should send a CV, bibliography, cover letter with a brief description of research experience and interests, and the names of 3 references (with phone numbers) to individual investigators listed below:

- Sohyun Ahn – Neurogenesis in the developing and mature nervous system of the mouse
- Harold Burgess – Analysis of neural circuits controlling behavior in zebrafish
- Mike Cashel – Molecular regulation of the prokaryotic stress response
- Ajay Chitnis - Early neural and lateral line development, Notch signaling
- David Clark – The role of chromatin structure in gene activation in yeast
- Robert Crouch – RNase H function in health and disease
- Igor Dawid – Early embryonic development and patterning in the frog and zebrafish
- Melvin DePamphilis - Regulation of DNA replication in normal and in cancer cells in the mammal
- Bruce Howard – Experimental and bioinformatic analysis of epigenome structure
- Judith Kassis – Gene silencing by Polycomb group genes (PcG) in *Drosophila*
- James Kennison – *Drosophila* developmental genetics
- Judith Levin – HIV-1 replication; reverse transcription, host restriction, and viral assembly
- Paul Love – Genes regulating mammalian hematopoiesis
- Richard Maraia – RNA metabolism, the La protein and RNA Polymerase III
- Keiko Ozato – Gene regulation in innate immunity
- Karl Pfeifer – Epigenetic regulation of gene expression in the mouse; mouse cardiac development and function
- Thomas Sargent – Epidermal and neural crest development in frog and fish
- Brant Weinstein – Vascular development in the zebrafish
- Heiner Westphal – Murine embryogenesis and induced pluripotent stem cells



**Department of Health and Human Services
National Institutes of Health
Medical Informatics Fellowships**

The Lister Hill National Center for Biomedical Communication (LHNCBC) at the National Library of Medicine seeks postdoctoral fellows as well as graduate and medical students, who are interested in collaborative research within a variety of biomedical informatics areas, including

- Capture, processing and analysis of clinical data for care and research applications
- Biomedical and document image analysis
- Development of health informatics resources.

Successful applicants are matched with LHNCBC staff and participate directly in ongoing research. LHNCBC's research activities include basic and applied research in fields such as:

- Tools and standards development for electronic medical records
- Natural language processing for understanding medical text and improving information retrieval
- Medical knowledge representation
- Text mining
- Multimedia database design
- Interactive publications
- Machine learning techniques
- Image processing research

LHNCBC has a tradition of advancing health information systems and its world class research staff is involved in activities that define and support the research infrastructure for next generation medical information systems.

Postdoctoral candidates should have a Ph.D., MD/OD/DDS or equivalent degree in medical informatics, information science, computer science, engineering, applied mathematics, or related disciplines. Candidates should have research experience in these areas. Medical student rotation programs are available as well as programs for graduate students. Post doctoral fellowships are in residence at LHNCBC in Bethesda, MD for one year with the possibility of renewal. Time in residence is variable for other awards, including visiting scholars, visiting faculty and graduate student candidates.

Stipends are commensurate with research experience and education. The annual application deadlines are: **January 15, March 15 and October 15**. However, applications also are considered year round under special circumstances. For additional information and instructions to submit an application, please see our **website: <http://lhncbc.nlm.nih.gov>**. The HHS and NIH are equal opportunity employers.

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UAB THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

ASSISTANT/ASSOCIATE PROFESSOR – CHEMISTRY – the Department of Chemistry at the University of Alabama at Birmingham seeks candidates for a tenure-track faculty position at the assistant or associate professor level in **CHEMISTRY** with research expertise in areas of chemical or biophysical applications of NMR and/or mass spectroscopy. Candidates whose research is complementary to existing strengths within the Department and School will be given preference. The University of Alabama at Birmingham (UAB) is a comprehensive research university and medical center with over 2,000 full-time faculty and over 17,000 students for Fall, 2010. UAB is ranked among the top tier research universities in terms of federal grant support. The Department of Chemistry offers B.S. (ACS-Certified), M.S., and Ph.D. degrees and has major research thrust areas in drug discovery, structural biochemistry, biophysical chemistry, computational chemistry, and polymer/advanced materials. Applications will be considered beginning **March 6, 2011**. Applications past that date will be considered until this position is filled. Candidates must have a Ph.D. degree in chemistry or biochemistry, postdoctoral or equivalent experience, and a commitment to teaching excellence at undergraduate and graduate levels.

Qualified applicants should send a letter indicating their interest, detailed curriculum vitae, description of research plans, a statement on their teaching experience and philosophy, and the names and contact information of a minimum of four references. At least one reference should be able to address your teaching potential, experience, and ability. Electronic submissions are encouraged and should be sent to **Ms. Laura Knighten (knighten@uab.edu)**, or mailed to the **Department of Chemistry, Faculty Search, University of Alabama at Birmingham, CHEM Suite 201, 1530 3rd Avenue South, Birmingham, AL 35294-1240**.

The Department of Chemistry and the University of Alabama at Birmingham are committed to building a culturally diverse workforce and strongly encourage applications from women and individuals from underrepresented groups. UAB has a Dual Career Assistance Program to support and offer resources to help spouses and partners of newly recruited UAB faculty. UAB is an Affirmative Action/Equal Employment Opportunity Employer.



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The selected applicant must have ability to obtain a DOE Q clearance, which typically requires U.S. Citizenship.

For more information or to apply, go to www.lanl.gov/jobs and reference job number **220659**.

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SALK INSTITUTE FOR BIOLOGICAL STUDIES

The Salk Institute for Biological Studies, a world class scientific environment located in La Jolla, CA, seeks candidates for faculty positions in the following areas:

JUNIOR OR SENIOR FACULTY POSITION IN THE WAITT ADVANCED BIOPHOTONICS CENTER

We are seeking a creative leader in biological imaging to establish an independent research program in our Waitt Advanced Biophotonics Center. The goal of this position is the development and application of new tools for transforming the study of biological processes.

TWO JUNIOR FACULTY POSITIONS IN NEUROSCIENCE

The successful candidates will be individuals with specific interests and expertise in studying 1) neural circuits and behavior using genetic approaches coupled with imaging and/or electrophysiology, and 2) synaptic physiology and dynamics. The Salk Institute maintains a world-renown program in the neurosciences with particular strengths in neuroendocrinology, vision, computational, developmental, behavioral and systems neuroscience.

The Salk Institute is a collaborative research environment that covers a broad range of topics including molecular biology, cellular biology, plant biology, developmental biology, neurobiology, systems biology, structural biology, and biological chemistry. We seek interactive individuals who are passionate in their pursuit of important problems in basic science.

The Salk Institute offers an excellent start-up package along with a competitive salary and benefits.

Qualified candidates are invited to submit their curriculum vitae, description of present and future scientific endeavors, and also arrange to have three letters of recommendation sent directly from the referees to: **biophotonics_search@salk.edu** (please reference job code: #A033) for the position in the Waitt Advanced Biophotonics Center OR **neuroscience_search@salk.edu** (please reference job code: #A034) for positions in Neuroscience.

Applications with all required materials will be reviewed beginning March 1, 2011 and will be accepted until positions are filled. Faculty positions available at the Salk Institute may be found at:

http://www.salk.edu/careers/faculty_positions.html.

The Salk Institute for Biological Studies is an Equal Opportunity Employer.



AAAS is here – connecting government to the scientific community.

As a part of its efforts to introduce fully open government, the White House is reaching out to the scientific community for a conversation around America's national scientific and technological priorities.

To enable the White House's dialogue with scientists, AAAS launched Expert Labs, under the direction of blogger and tech guru Anil Dash. Expert Labs is building online tools that allow government agencies to ask questions of the scientific community and then sort and rank the answers they receive.

On April 12, 2010, AAAS asked scientists everywhere to submit their ideas to the Obama administration and at the same time launched the first of Expert Labs tools, Think Tank, to help policy makers collect the subsequent responses. The result was thousands of responses to the White House's request, many of which are already under consideration by the Office of Science and Technology Policy.

As a AAAS member, your dues support our efforts to help government base policy on direct feedback from the scientific community. If you are not already a member, join us. Together we can make a difference.

To learn more, visit aaas.org/plusyou/expertlabs





AAAS is here – promoting universal science literacy.

In 1985, AAAS founded Project 2061 with the goal of helping all Americans become literate in science, mathematics, and technology. With its landmark publications *Science for All Americans* and *Benchmarks for Science Literacy*, Project 2061 set out recommendations for what all students should know and be able to do in science, mathematics, and technology by the time they graduate from high school. Today, many of the state standards in the United States have drawn their content from Project 2061.

Every day Project 2061 staff use their expertise as teachers, researchers, and scientists to evaluate textbooks and assessments, create conceptual strand maps for educators, produce groundbreaking research and innovative books, CD-ROMs, and professional development workshops for educators, all in the service of achieving our goal of universal science literacy.

As a AAAS member, your dues help support Project 2061 as it works to improve science education. If you are not yet a AAAS member, join us. Together we can make a difference.

To learn more, visit aaas.org/plusyou/project2061



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University of Massachusetts Amherst

The Graduate Program in Organismic and Evolutionary Biology (OEB) at University of Massachusetts Amherst announces a two-year postdoctoral fellowship/lectureship. OEB draws together more than 80 faculty from the Five Colleges (University of Massachusetts Amherst and Smith, Hampshire, Mount Holyoke, and Amherst Colleges), offering unique training and research opportunities in the fields of ecology, organismic, and evolutionary biology. Our research/lecture position provides recent Ph.D.'s with an opportunity for independent research with an OEB faculty sponsor as well as experience developing and teaching a one-semester undergraduate biology course. Proven teaching skills are required. Position subject to availability of funds. First-year salary: \$35,000. Second-year salary: \$37,000.

To apply, send curriculum vitae, three letters of reference, statements of research and teaching interests, and arrange for a letter of support from your proposed OEB faculty sponsor. A list of faculty and additional information is available at [website: http://www.bio.umass.edu/oeb](http://www.bio.umass.edu/oeb).

OEB Darwin Fellowship
319 Morrill Science Center
611 N. Pleasant Street

University of Massachusetts Amherst
Amherst, MA 01003

Telephone: 413-545-0928

E-mail: darwin@bio.umass.edu

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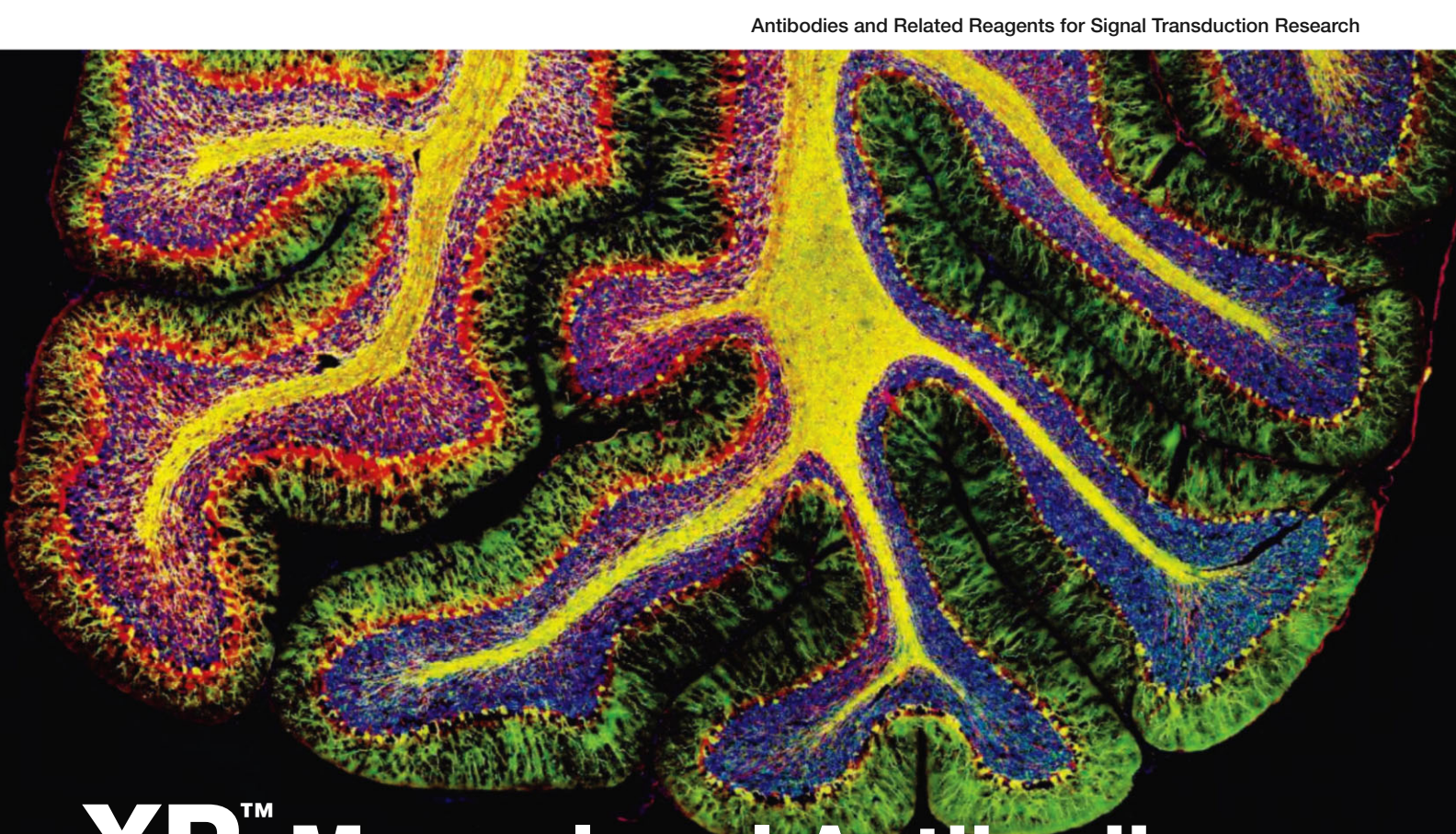
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